



*The Pathology of*

# DIABETES MELLITUS

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BY

**Shields Warren, M.D., ScD.**

Departments of Pathology of the New England Deaconess Hospital and the Harvard Medical School Boston Massachusetts Director of the Division of Biology and Medicine U. S. Atomic Energy Commission

AND

**Philip M. LeCompte, M.D.**

Departments of Pathology of the Faulkner Hospital and the Harvard Medical School Boston Massachusetts

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## PREFACE

THE abnormalities of function of vital importance to the diabetic patient are intimately related to changes in cell and tissue structure. As new techniques in histochemistry and histology have been developed our ability to relate and understand these structural and functional alterations has been materially increased. Our knowledge of diabetes has been far from static since 1938 and many new factors have been developed. This third edition is larger than previous editions in spite of elimination of much old material as a result of the continuing advances that have been made in the field. These advances are particularly important in the field of experimental diabetes in the understanding of the changes encountered in infancy and childhood and in special pathology particularly that of the kidney, the eye and endocrine glands.

No one man's knowledge can hope to be complete in this field and the authors are deeply indebted to many for help in varying degree. Without the continuous stimulation of Dr Elliott P Joslin the many obstacles incident to a task such as the preparation of this book would have been even more obvious. Drs Alexander Marble, Howard A Root, Priscilla White, Francis D W Lukens and Stanley Robbins have been most helpful and have read one or more chapters. Valuable material or helpful suggestions have been received from Drs David Cogan, Arnold Izzirow, H Berning, Jonas S Rudenwald, G Lyman Duff, G Gomori, F I Bell, I I Stubbs, Frank Bloom, J B Hazard and G Kenneth Muller. I urge sincerely without the aid by the help of those working with us in the technical and secretarial fields the preparation of the volume would have been extremely difficult. We are indebted to Miss Nancy Buck, Mrs Diane Crocker and Mrs Mildred Reilly for histologic technical assistance to Miss Jane Hennelly for compilation of tables to Mrs Kathryn Haley for photomicrographs and proofreading and to Mrs Jean LeCompte for assistance in the preparation of the manuscript and proofreading.

SHELDON WARREN

PHILIP M Lecompte





# CONTENTS

CHAPTER	PAGE
1 Diabetes Mellitus and the Pancreas	7
2 The Normal Pancreas	11
3 The Pancreas in Diabetes Mellitus	11
4 Lesions of the Pancreas in Nondiabetic Individuals	76
5 Pathological Evidence of Abnormal Carbohydrate Metabolism	82
6 Pathological Evidence of Abnormal Fat Metabolism	102
7 The Pathology of Acidosis and Coma	116
8 Diabetes and Infection	122
9 Vascular Disease in Diabetes	129
10 Gangrene	131
11 The Heart	137
12 The Kidney	168
13 The Eye	181
14 The Nervous System	191
15 The Pituitary	191
16 The Adrenal Glands	201
17 Other Endocrine Glands	206
18 The Pathology of Diabetes in Children	210
19 The Pathology of Diabetes in Cases of Long Duration	216
20 Hemochromatosis	226
21 The Pathology of Insulin Resistant Cases	231
22 Hyperuricemia and the Pathological Effects of Insulin	240
23 Infants of Diabetic Mothers	248
24 Cancer and Diabetes	268
25 Experimental Diabetes	271
26 The Etiology and Pathogenesis of Diabetes	290
27 Medical-Legal Aspects of Diabetes Mellitus	310
28 A Unifying Concept of the Pathology of Diabetes	316



# THE PATHOLOGY OF DIABETES MELLITUS

## Chapter 1

### DIABETES MELLITUS AND THE PANCREAS

The name insulin given the pancreatic hormone has by suggestion so thoroughly convinced us of the truth of the isular hypothesis that it is well to examine briefly the evidence upon which it rests at the present time and to see whether we are justified in accepting it in its entirety. The importance of the truth of this theory with regard to the etiology, treatment and prognosis of diabetes mellitus is obvious. Although Cawley<sup>1</sup> in 1768 reported a case of diabetes that came to autopsy and showed marked pancreatic damage (the pancreas was full of calculi which were firmly impinged in its substance, the right extremity of the pancreas was firmly and appeared to be scarious) the relation of the pancreas to diabetes was not recognized for another century. A few scattered observations<sup>2,3</sup> suggested the importance of this gland in diabetes but failed to attract any general attention. Only with the discovery of von Mering and Minkowski<sup>4</sup> that removal of the pancreas would cause diabetes in experimental animals was the relationship definitely established. Even so the occurrence of apparently normal glands in many diabetics and the absence of diabetes in individuals much of whose gland had been destroyed by necrosis or by malignant disease were thorns in the flesh of the pathologists for twenty years and even now there are twinges from the wound. Moreover, Minkowski's experiments showed that the presence of only one-eighth of the pancreas was sufficient to avert the development of diabetes in animals so that the pancreatic lesions found in human cases of diabetes hardly seemed sufficiently severe.

Laguesse<sup>5</sup> hazarded the opinion that the islands of the pancreas were regions of internal secretion basing this on anatomical grounds especially the relation of the epithelium of the islands to the rich vascular network that surrounds it. The first extensive pathological study was that of Hansmann<sup>6</sup> in 1884 who collected 72 cases from the literature and presented a new case. However this group was studied chiefly from the standpoint of gross pathology. Hansmann's tables are given below.

The granular atrophy of Hansmann is identical with Opie's intracinar fibrosis. It is of more than passing interest that the islands of Langerhans are not mentioned in Hansmann's tables. In brief Hansmann was the first as well as the chief champion of the isular hypothesis assuming that

TABLE 1

<i>Cases from the Literature</i>		<i>Cases from the Berlin Pathological Institute</i>	
Simple atrophy	16	Granular atrophy	36
Chronic interstitial inflammation	18	Fibrous induration with hypertrophy	3
Pancreatic calculi	15	Complicated case	1
Necrosis (? of post-mortem change)	6	Normal pancreas	8
Carcinoma	5	Condition not noted	6
Lipomatous	3		
Fatty degeneration	3		
Abscess	2		
Cyst	1		
Hyperemia	1		
Total	72	Total	54

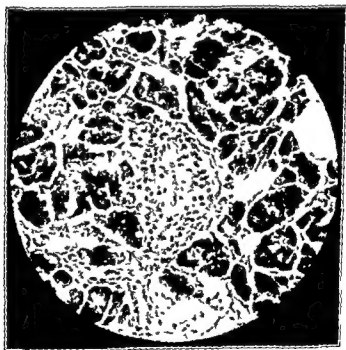


FIG. 1.—Moderate hyalinization of island of Langerhans. Male, aged sixty-six years.  
Duration of diabetes fourteen years.

the acini were the seat of control of carbohydrate metabolism and consistently opposing the insular hypothesis.

The transplantation of the remaining portions of partially resected pancreases by Hédén<sup>7</sup> with the failure of diabetes to develop as long as the transplants remained in healthy condition served to show that an internal secretion and not the external was concerned in carbohydrate metabolism. The observation of Opie,<sup>8,9</sup> later confirmed by Sobolew<sup>10</sup> localized in the islands the pathological changes associated with diabetes and explained at least in part the previous difficulties in correlation of the clinical and pathological findings. In cases of diabetes mellitus the islands were frequently diseased with or without damage of the acinar tissue while in cases of extensive pancreatic lesions with normal carbohydrate metabolism such as some cases of acute pancreatic necrosis or carcinoma the islands were found to be intact. The abnormalities in the islands were either qualitative as sclerosis or hyalinization or else quantitative. Sobolew described complete absence of islands in 4 out of 13 cases of diabetes. This finding is most remarkable. Probably if he had had the advantage of the specific staining methods for the island cells discovered by Lane,<sup>11</sup> Bensley,<sup>12</sup> and Gomori,<sup>13,14</sup> he would have found at least some island tissue present perhaps in the form of islet cells scattered through the acinar tissue as sometimes occurs.

Another earlier report<sup>5</sup> of absence of islands is uncertain as the author failed to find islands in two nondiabetic cases as well as the one of diabetes. Likewise Binger and Keith<sup>15</sup> reported a case of apparent complete absence of islet tissue in a nondiabetic. No instance of complete absence of insular tissue checked by specific staining methods has been reported.

In 1904 Sauerbeck<sup>17</sup> analyzing the available evidence championed the insular hypothesis and it has remained the favorite up to the present. Opie<sup>18</sup> presents a table (Table 2) based on cases compiled by Sauerbeck and himself.

Heiberg<sup>19</sup> and Weichselbaum<sup>20,21</sup> each claimed pancreatic changes to be present in every case of diabetes and to be limited to the islands in a sufficient number of cases to prove the insular hypothesis. Cecil<sup>22</sup> in a series of 90 cases came to the same conclusion and Litter<sup>23</sup> called attention to the frequent occurrence of evidence of regeneration or hypertrophy in the islands of Langerhans in diabetes (34 out of 100 cases). Similar changes suggesting hypertrophy and regeneration were found in cases of chronic pancreatitis without diabetes. Yotsumugi<sup>24</sup> has described new formation of islands from centro-acinar cells as well as regenerative hypertrophy in cases of severe pancreatic damage.

The presence of regenerative processes in the islands was also stressed by Weichselbaum<sup>25</sup>—in cellular proliferation of the island epithelium and the formation of new islands from the epithelium of the ducts. With all this evidence favoring the insular hypothesis there was nevertheless an undercurrent of discouragement or dissatisfaction with the available means

TABLE 2 — PANCREATIC LESIONS SHOWN BY CASES COMPILED BY SALKERBLECK AND OPIE

Interacinar pancreatitis	125
Interlobular pancreatitis	14
Lipomatosis	18
Calculi	9
Cyst	1
Carcinoma	5
Focal necrosis	2
Atrophy	65
Lesions of islands with normal parenchyma	
Hyaline degeneration	6
Sclerosis	2
Adenoma like hypertrophy	1
No lesion of pancreas	
Pancreas normal	14
Number of islands diminished	3
Total	298

of recognizing characteristic lesions in diabetes. This feeling was well expressed by Allen<sup>26</sup> nearly forty years ago and his statement even today holds true. Every anatomical hypothesis of diabetes still requires to be assisted by assuming the existence of a certain proportion of functional cases without known anatomical basis.

With the differentiation by Lane<sup>11</sup> of  $\alpha$  and  $\beta$  cells in the islands of Langerhans and with the beautiful studies of Bensley<sup>12</sup> on the pancreas of the guinea pig it was natural to attempt to correlate cytological with clinical findings in the hope that there might be found some single universal diabetic lesion. Homans<sup>27</sup> found disappearance of the secretory granules in the island cells of rats from which three-fourths of the pancreas had been removed. In those animals which became diabetic he noted hydropic degeneration of the island cells and hence regarded them as the site of the carbohydrate-regulating function. This hydropic degeneration previously noted by Weischelbaum<sup>28</sup> in diabetic patients and which Allen<sup>26</sup> had produced experimentally in dogs. Homans<sup>28</sup> found to affect chiefly the  $\beta$  cells.

This gave new hope for the existence of a single characteristic universal diabetic lesion. Further study has unfortunately blasted this hope. Hydropic change of the  $\beta$  cells may well mean diabetes when it occurs but by no means all diabetic pancreases show this lesion.

In recent years considerable interest has been shown in the quantitative relationships between the two major types of islet cells. As will be noted in Chapter 3 differential counts of the ratio of  $\beta$  to  $\alpha$  cells by various more or less specific staining methods have led to conflicting results some observers (Ferner<sup>29</sup> Hess<sup>30</sup>) claiming to find a striking and diagnostic reduction in the ratio others (Gomori<sup>31</sup> & Terbruggen<sup>32</sup>) finding in many cases ratios either entirely within normal limits or showing only slight deviation.

The duct ligation experiments of Schulze<sup>21</sup> Ullar<sup>25</sup> and others<sup>26, 27</sup> showed that extensive (though now known to be incomplete) atrophy of the acinar tissue was unaccompanied by disturbance of carbohydrate metabolism.

Insulin<sup>28</sup> was the climax of this line of experimentation. In spite of the important evidence adduced by the production of insulin from the principal islets of fish<sup>29, 30</sup> and from pancreatic tissue in which the acini had practically disappeared secondary to duct ligation, the production of insulin in the entire absence of acini was not yet proved.

It remained for Wilder's<sup>31</sup> striking case of hyperinsulinism due to carcinoma of the islands of Langerhans to clinch the truth of the insular hypothesis. This was further reinforced by the carcinoma of Thalhimer and Murphy,<sup>32</sup> the adenoma of McClenahan and Norris<sup>33</sup> and by the results of the operative removal of the carcinoma of island-cell type reported by Howland.<sup>34</sup>

Of some interest in this connection are cases of naturally occurring diabetes in animals. Spontaneous diabetes in dogs has been reported by Bloom and Handelsman<sup>35</sup> and Bloom has observed spontaneous diabetes associated with hyaline islets in the cat.<sup>36</sup> More recently Landé<sup>37</sup> has described the case of a four-year-old cat which developed thirst and excessive urination and at autopsy showed small islets with some hydropic and myxomatous changes. In the case of Pollock and Bauman<sup>38</sup> a six-year-old Irish setter exhibited polydipsia, polyuria and weight loss and at autopsy showed an enlarged pancreas (65 gm.) in which the islets were

we found a peculiar massive coagulative necrosis of the pancreas and a liver almost replaced by fat. According to Bloom (personal communication) a fatty liver is characteristically found in both canine and feline diabetes. (Certainly, as noted by Pollock and Bauman<sup>38</sup> more attention should be given to these interesting cases.)

Schittauer and Villar<sup>39</sup> report 9 cases in dogs and cats and note that the disease may be due to pancreatitis or may be idiopathic. They illustrate hyaline islets in one cat and state that most diabetic dogs are females and most diabetic cats are males.

According to Dr. I. I. Stubbs<sup>40</sup> the incidence of diabetes in domestic animals as judged from various series reported in the literature is probably highest in dogs but even here is quite low—most authors giving figures of less than  $\frac{1}{4}$  of 1 per cent and many as low as 1 in 10,000. He also cites the interesting statement of Runnells<sup>41</sup> that diabetes is believed to be the cause of death in feeder lambs when overfed on carbohydrates such as corn sugar molasses. If this conclusion be true the important implications in connection with the experimental work of Dohan and Lukens<sup>42</sup> and the role of diet are obvious (Chapters 25 and 26).



Perhaps the most convincing and dramatic evidence in favor of the insular hypothesis is the remarkable specific damaging effect of alloxan on the  $\beta$  cells of the islets and the intimate correlation between this damage and the development of diabetes in animals so treated (*see* Chapter 25). Other bits of evidence include the correlation of lowered insulin content of the pancreas with injury to the islets<sup>42</sup> the lesions of the islets in pituitary diabetes<sup>43</sup> the degranulation of the  $\beta$  cells in experimental hyperglycemia<sup>42-44</sup> and in human diabetes<sup>45</sup> the changes of the mass of islet tissue in response to treatment with glucose or with insulin<sup>47</sup> and the fact that diabetics excrete less insulin in the urine than do nondiabetics.<sup>48</sup>

### THE ACINO INSULAR HYPOTHESIS

That the malfunction of the pancreas as a whole is responsible for diabetes is the essence of the acino insular hypothesis. Lombroso<sup>49</sup> presented evidence for this theory. In the hands of some other proponents such as Reitmann<sup>50</sup> who believed that normally islands and acini both controlled carbohydrate metabolism yet either alone could perform this function and Karakascheff<sup>51</sup> who believed the islands were only of use to replace worn-out acini and were not separate organs this theory was decidedly weak. While Laguesse<sup>52</sup> valiantly upheld the possibility of reciprocal transitions between acini and islands he nevertheless believed the islands to be primarily concerned with carbohydrate metabolism. This hypothesis is a logical corollary of the transition theory of interrelation of islands and acini which will be discussed in Chapter 2.

A monograph of Seyfarth<sup>53</sup> sums up the evidence and perhaps marks the height of the prestige of this theory. Gomori<sup>54</sup> has summarized the points of view of the two opposing schools of thought in relation to the interdependence of acinar and islet tissue and has presented his own reasons for doubting the supposed conversion of acinar cells into islet cells and vice versa.

### THE ACINAR HYPOTHESIS

Of historical interest alone is the belief of Hansmann<sup>5</sup> that the acinar cells are the seat of regulations of carbohydrate metabolism. He laid great stress on granular atrophy essentially interacinar fibrosis as the most important lesion in diabetes.

### DEFECTS IN THE PANCREATIC THEORY

In recent years there has been increasing realization that the whole picture of human diabetes cannot be interpreted solely in terms of the pancreas and much has been made of the fact that many cases of the dis-

diabetes due to adrenal tumors have been permanently cured by removal of the tumors<sup>66</sup> and finally that total pancreatectomy in man results in a relatively mild form of diabetes<sup>67 68</sup> On the other hand it must be recognized that all known forms of *permanent* experimental diabetes are associated with lesions of the islets and that probably the great majority of human cases would show lesions if studied with proper histologic methods and quantitative techniques. It is interesting that such a prominent authority as Wilder<sup>69</sup> in a recent important paper offers a spirited defense of the unitary pancreatic view of human diabetes. Perhaps the soundest point of view is to regard the majority of cases as fundamentally pancreatic (though modified by various factors) and to recognize a small group of definitely extra pancreatic origin based perhaps primarily on dysfunction of the liver or adrenals. If one adopts the point of view of Humsworth<sup>70</sup> and regards diabetes mellitus as a *syndrome* rather than a disease entity much of the difficulty of thinking of it in anatomical terms is obviated (see Chapter 26).

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## Chapter 2

# THE NORMAL PANCREAS

The relation of the pancreas—particularly its islets—to carbohydrate metabolism is so marked as to necessitate a thorough understanding of the normal histology of the gland in any consideration of the anatomical pathology of diabetes mellitus. Few organs of the body are less constant in size and architecture than the pancreas. The descriptions given in most textbooks of anatomy cover the essential features of the gland but give altogether too great an impression of its constancy in morphology. For example the weight at autopsy in individuals showing no evidence of pancreatic disorder ranges from 60 to 160 grams averaging about 95 grams. One of the best descriptions of the pancreas is that of Opie<sup>1</sup> and this has been freely drawn upon in the present discussion.

In the loop of the duodenum lies the head of the pancreas. From the upper and anterior aspect of the head a constricted portion, the neck, bends slightly upward and forward and then stretches to the left forming the body of the gland. Behind the neck pass the superior mesenteric vessels. The body tapers slightly toward the spleen and this thinner portion of the gland is called the tail.

The development of the pancreas takes place from three primitive outgrowths from the mid gut, one of which is on the dorsal surface between the liver and the stomach and the other two a right and a left ventral which subsequently fuse and rise on either side of the hepatic duct and then grow to meet the dorsal part. With the alteration in position of the stomach and duodenum the pancreas is changed in position so that the dorsal portion lies transversely with its left extremity near the spleen and the ventral portion is carried with the torsion of the duodenum to the right and backward. Thus with further growth the part of the pancreas which was originally ventral comes in contact with the larger dorsal part and unites with it as a rule during the second month of embryonic life.

In studies on 21 human embryos Pearse<sup>2</sup> found the islets to appear first in the 54 mm embryo as oval eosinophilic cell masses continuous with the acini. During the third month of development a few completely separated islands were found while most remained connected with the acini by solid stalks of cells. Each island was provided with four to seven afferent and efferent capillaries. Development is not infrequently arrested in congenital syphilitic pancreatitis and islands are found still communicating with acini and ducts. In light of the studies of Bensley and others the

islands probably have a common origin with the acini from the duct cell rather than arising from the acinar cells. However, the acino-insular relationship is still disputed.<sup>2</sup> Neubert<sup>4</sup> in a detailed study of the development of the pancreas held that the islets arise from the secreting ends of the adenomeres as well as from the ducts. This extensively quoted work is considered by Krus<sup>5</sup> and others to offer a reasonable explanation of the acino-insular relationship and at least to imply the possibility of new formation of islets from acinar tissue in the adult. Hughes<sup>6</sup> from a study of alloxan diabetes holds that formation of islet cells from acini occurs in the rat (see page 27 for further discussion). Gomori<sup>7</sup> points out that much of the evidence for transformation of acinar cells to islet cells is based on insufficient evidence or inadequate technique.

Lerner<sup>7</sup> has worked extensively with the Gros-Schultze method of silver impregnation and has studied the islets at all stages of development. He holds that the silver positive cells include the  $\alpha$  cells of the islets and in addition cells scattered singly and in groups among the acini in the connective tissue and in the ducts. In the embryo he describes buds of these silver cells arising from both ducts and acini and refers to them as being *inselpotente* i.e. the precursors of islets. In early developing islets he describes the appearance of a central nucleus of  $\beta$  cells which do not take the silver surrounded by the unripe silver positive cells. The silver cells predominate in early life in fact are exceedingly numerous at birth then decrease relative to the  $\beta$  cells through at about the fourth year after which time the relationship becomes more or less stabilized. According to this point of view the silver cells including the  $\alpha$  cells are resting non-functional elements from which  $\beta$  cells may be formed under the proper stimulation. Further discussion of Lerner's ideas is given later in this chapter and in Chapter 3.

Hard<sup>8</sup> working with rat embryos and using the neutral gentian method of Bensley and Gomori's modification of the Mallory Heidenhain stain found that the  $\beta$  cell was the only islet cell type to differentiate during embryonic life and that the specific granules could be distinguished at about the nineteenth day i.e. fairly late in fetal life. The  $\alpha$  cell (rather surprisingly in view of Lerner's statements noted above) was not demonstrable at all during fetal life being recognized first on the second postnatal day.

The pancreas is traversed from left to right by the duct of Wirsung which bends downward and backward in the neck and after traversing the posterior surface of the head reaches the wall of the duodenum where it unites with the common bile duct. The accessory pancreatic duct that of Santorini arises in the upper portion of the head and terminates in a small papilla in the mucous membrane of the second portion of the duodenum about 2 cm. above the papilla of Vater.

There are numerous variations in the distribution of the ducts and their anastomoses. In 100 pancreases Opie<sup>9</sup> found the ducts in anastomosis in

90 He gives a number of excellent diagrams of the relationships of the two ducts

On the surface of the pancreas irregular areas are demarcated by loose connective tissue and fat. The amount of this fat varies greatly with the state of nutrition of the individual and is one of the chief factors in the variability of weight of the gland. The gland can be divided by dissection of the tissue into clear cut lobules usually about 1 cm in diameter. These primary lobules are subdivided into smaller secondary lobules usually several millimeters in diameter by poorly-defined septa. With the low power of the microscope these secondary lobules may be still further divided to tertiary lobules.

Aberrant pancreatic tissue may occur anywhere in the upper gastrointestinal tract. Faust and Mudgett<sup>9</sup> review the literature and report a case in which a mass of pancreatic tissue presented as a polyp of the stomach.

The blood supply of the body and the tail of the pancreas is from branches of the splenic artery, where the head receives its supply from the superior pancreaticoduodenal artery, a branch of the hepatic and from the inferior pancreaticoduodenal artery, a branch of the superior mesenteric. The bulk of the venous blood from the pancreas passes into the splenic vein and a smaller amount into branches of the superior mesenteric vein. Anastomoses are frequent both arterial and venous. An excellent review is given by Kirk.<sup>10</sup>

The gland is innervated by fibers from the lienal plexus which in turn is formed by branches from the left celiac ganglion, the celiac plexus and right vagus nerve. Pacinian corpuscles are not rare. Sympathetic ganglion cells may be found though rarely in all parts of the pancreas.

## THE HISTOLOGY OF THE PANCREAS

The following description of the pancreatic cells is based on the study of human autopsy material fixed from twenty minutes to three hours post mortem. No pancreas showing gross evidence of pathological change was included. In addition the information disclosed by the far more careful researches of Bensley,<sup>11</sup> Laquessa,<sup>12</sup> Ullrich<sup>13</sup> and many others has been liberally drawn upon.

Supposedly normal pancreatic tissue was fixed routinely in Zenker's fluid, sublimate-bichrome etc. and formalin. Frequently Bouin's fluid, Champy's fluid, Zenker formal acetocarmum-bichromate, osmic acid, absolute alcohol and Regaud's method were also resorted to.

The following stains were used:

Phloxine-methylene blue

Phosphotungstic acid hematoxylin

Mallory's aniline blue

Carpenter's eosin-methylene blue

Masson's iron hematoxylin vert lumière

Iron hematoxylin Van Gieson

Ukai's stain

Bensley's neutral gentian

Gomori's chrome-alum hematoxylin and phloxine

Gomori's new aldehyde-fuchsin method

Gros-Schultze silver method (slightly modified from Ferner)

On certain favorable cases various lipid stains were employed as were Best's and Langhans' methods for glycogen. When the tissue was fresh and properly fixed the aniline fuchsin methyl green or picric acid method or the iron hematoxylin method for mitochondria was employed as were Bensley's safranin stain and a very useful modification of Mallory's aniline blue stain devised by Bensley.<sup>14</sup>

Recently we have had considerable success with Gomori's chrome-alum hematoxylin and aldehyde-fuchsin methods perhaps the most generally useful stains for the islets yet devised. On well fixed material his modification of the Mallory-Heidenhain azocarmine method is also of value. Also we have made extensive trials of Ferner's<sup>17</sup> method of applying the Gros-Schultze silver stain to the pancreas and have had good results with a slight modification of it. This may yet prove to be the most useful method for estimating in autopsy material the relative numbers of  $\alpha$  and  $\beta$  cells. Unfortunately it only works well on frozen or celloidin sections. Our efforts to develop a workable silver method for paraffin sections have met with limited success.

Details of the more useful methods are given in Appendix A. A critical discussion is given by Gomori.<sup>2</sup>

**Duct Cells.** The simplest system in the pancreas is that of the ducts with which may be included the centro-acinar cells. Moreover from the primitive duct epithelium the external and the internal secretory elements of the gland are derived. As would be expected from their origin from the duodenal epithelium the ducts at their proximal ends are lined by epithelium closely resembling that of the duodenum—tall columnar cells with distally placed elongate nuclei. Granules varying in size which give the characteristic staining reactions of mucin occasionally occur in the proximal portion. True goblet cells occur with varying frequency. Near the duo-

At times even the neoplasms derived from the two are practically indis-

The mucous secretion of the cells disappears and the epithelium gradually changes from columnar to cuboidal as the ducts become finer. There is a tendency for the duct epithelium to flatten slightly in the intercalated ducts and frequently the centro-acinar cells flatten very abruptly. The centro-acinar cells are similar in appearance to the cells of the finer ducts and probably represent the termini of the ducts. They tend to insinuate themselves about the apices of the acinar cells.

Jacoby<sup>10</sup> demonstrated alkaline phosphatase in the duct epithelium by histochemical methods and postulated that the ductules were the source of this enzyme, the other three enzymes of the pancreatic juice being produced by the acini. Wang, Grossman and Ivy<sup>11</sup> confirmed the histochemical observations and showed further that pancreozymin stimulates the output of amylase from the pancreas but not that of alkaline phosphatase.

Both duct cells and centro-acinar cells have a clear, acicellular cytoplasm free from secretory granules and chromidial substance. In favorable cases scattered fine rod-shaped mitochondria are visible in both duct cells and centro-acinar cells. Nassimov<sup>12</sup> demonstrated a simple Golgi apparatus in these cells. It is much less complicated than in the acinar and island cells.

The nucleus is usually oval, smaller than that of the acinar cell and relatively rich in chromatin. The nucleolus, so prominent in the acinar cell, is not obvious. Extremely rarely mitosis is seen even in adults.

Bensley<sup>13</sup> has demonstrated in the guinea pig, through vital staining with pyronin, a system of fine anastomosing tubules surrounding the larger ducts and communicating with the ducts on the one hand and the islands of Langerhans on the other. Moreover scattered in these tubules are single cells identical with those of the islands. Otani<sup>14</sup> found in human cases direct connection between a moderate proportion of the island and the interlobular or intralobular ducts.

**Acinar Cells** — That portion of the pancreas with which we are best acquainted is the external secretory system. The obvious importance of the pancreatic ferments and the striking appearance of the acinar cells early focussed attention on the cytology of the latter. The easy demonstration of the zymogen granules made attempts at correlation between changes in the intracellular structure and glandular secretion peculiarly enticing.

With any of the ordinary stains the cytoplasm of the acinar cells is clearly divided into two zones. The inner zone is markedly vacuolated and with appropriate staining these vacuoles are seen to contain, or rather be, the zymogen granules. The outer portion of the cell usually containing the nucleus, stains strongly with basic dyes and is faintly striated. Thus when stained with Goudpasture's methylene blue after chromic-sulphuric or Zanker-formol fixation, the outer portion of the cell shows a diffuse bluish coloring (chromidial substance) with faint clear striations. These striations are known to be due in animals to the presence of mitochondria. While we have had great difficulty demonstrating mitochondria in human pancreatic



tissue in several cases they have had this same distribution. Acetic or osmic acid in the fixing fluid causes the chromidial substance to be precipitated in filamentous form (Bensley<sup>11</sup>). However, Saguchi<sup>12</sup> believes the filaments are in reality preformed and simply made visible by the acid.

In the human cases in which mitochondria are found they appear as fairly long slightly wavy rods of moderately irregular thickness arranged more or less parallel to the long axis of the cell and only very rarely occurring in the inner or vacuolated zone. We have not been able to detect any definite relation between mitochondria and zymogen granules.

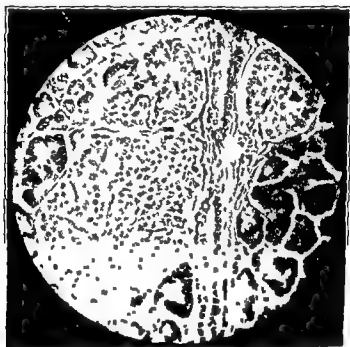


FIG. 2. Normal pancreas showing acini island and a duct. Male age 140 days. No label. Phosphotungstic acid hematoxylin stain.  $\times 170$ .

With stains such as phosphotungstic acid hematoxylin following formalin fixation or as Bensley's neutral gentian the zymogen granules are the most prominent elements of the cell. These granules vary in size but in fairly close range. Their origin is still in dispute being considered as mitochondrial derivatives, products of the Golgi apparatus or of the chromidial substance. Even the nucleus has been thought their source.

Occasionally there are scattered foci of cells ranging from a portion of an acinus to several adjacent acini in size which stand out markedly acidophilic with eosin-methylene blue staining. These cells have a clear homogeneous cytoplasm and apparently do not contain either zymogen

granules or chromidial substance. These are more likely foci of exhausted cells rather than foci of necrosis. They throw some light on the supposed transition of acinar to island tissue as in casual examination of a section they strongly resemble islands. However, these cells never contain the specific granules of the island cells and their arrangement is in no way characteristic of islands. They are presumably identical with the groups of eosinophilic cells described by Hamperl<sup>20</sup> in various organs especially salivary glands parathyroid and thyroid and called by him onkocytes. Curiously, Hamperl does not mention the pancreas in his latest paper<sup>9</sup> on 'onkocytes' although previously<sup>21</sup> he placed some emphasis upon it as a common site of these cells. At any rate it would seem that little is to be gained by labeling these cells since by so doing no light is cast upon their nature or function.

**Island Cells.**—Distributed irregularly in the pancreatic parenchyma are the groups of small polygonal cells with homogeneous cytoplasm first described by Langerhans.<sup>22</sup> These cell groups vary greatly in size but most are between 75 and 175 micra in diameter. The variation in number is also great. Bensley<sup>23</sup> has counted as many as 50,000 in the pancreas of the guinea pig and found an average of 22.3 per cmm in adult guinea pigs. Clark<sup>24</sup> found from 250,000 to 1,750,000 in the pancreases of non-diabetic adult humans. Owing to variation in distribution even in the same pancreas estimates of the number of islands present are of but little value. They are said to be more frequent in the tail than in the remainder of the organ.

A quantitative study of the pancreatic islands was made by Ogilvie.<sup>25</sup> He used an ingenious method for calculating the area and weight of the islets by projecting microscopic fields on a sheet of paper tracing the outlines of the islets weighing both the intact sheet and the cut-out islets calculating the volume by assuming the average islet to be a sphere and finally applying a formula to calculate the weight of islet tissue in relation to the weight of the pancreas. Forty-five fields were examined from each of 70 pancreases from females and 41 from males ranging from newborn to sixty-four years of age. The average weight of the pancreas increased from 2.0 grams at birth to 6.0 grams for those over twenty-one years of age while the average total weight of the islands increased from 0.12 grams at birth to 1.07 grams for those over twenty-one years of age. However the insular weight varies through a wider range than does the total pancreatic weight. For the first two years the rates of increase of islands and of total pancreas are parallel and greater than the rate of body growth. From four to twelve years of age the rate of increase of islet tissue is about one-half that of the total pancreas and the body as a whole but at adolescence the rates are again equal.

After the third year Ogilvie found the number of islands to remain fairly constant the weight of the average island increasing from 0.30 mg. at birth to 1.46 mg. in adult life. The weight varies over a wide range even in adults.

from 0.478 $\gamma$  to 2.738 $\gamma$ . When the islands are relatively few, or when they are more numerous than average they tend also to be small. Larger

117.226 in a year-old

Many pancreases in adult life contain about 1,000,000 islands, but the figures vary nearly as widely as those just given. Heiberg<sup>25</sup> estimated that the islands made up approximately 3 per cent of the pancreatic tissue. Bargmann<sup>26</sup> gave counts in 5 adults varying from 208,369 to 1,760,000.

The most careful quantitative study of the islands of Langerhans is that of Tejning<sup>27</sup> who followed the effect of diet on the volume of the islet organ in rats. He used serial celloidin sections 30 micra thick of the carefully weighed formalin fixed pancreas projecting and tracing the islets in every tenth section and applying the mathematical methods of Wicksell for calculating the volume of ellipsoidal solids. He found a definite correlation between the total volume of the islets and the numerical and volumetric distribution of islets of different magnitude. He also found a positive correlation between the size of the islet organ and the pituitary and a sex difference in that the female rats tended to have a larger islet organ both on normal and special diets. His results on the size of the islet organ as influenced by various diets are of great interest and are summarized in the accompanying table.

TABLE 3 (FROM TEJNING<sup>27</sup>) VOLUME OF ISLET TISSUE OF RATS EXPRESSED IN CM<sup>3</sup> PER 100 GM BODY WEIGHT

	High Carbohydrate	High Protein	High Fat	Natural Diet
Males	0.711 $\pm$ 0.022	0.594 $\pm$ 0.028	0.412 $\pm$ 0.021	0.673 $\pm$ 0.026
Females	0.880 $\pm$ 0.028	0.684 $\pm$ 0.030	0.504 $\pm$ 0.023	0.442 $\pm$ 0.020

Tejning<sup>27</sup> has criticized (and apparently with some validity) the work of all previous investigators in the quantitative field including Bensley<sup>11</sup> and Ogilvie<sup>12</sup> as involving technical errors, inadequately controlled animal material or unjustified mathematical assumptions. For the details of his arguments and methods reference should be made to his paper. He has undoubtedly made a valuable contribution in his critical survey of the field and his emphasis on the extreme care that must be exercised in order to obtain valid results in this difficult line of investigation.

Methods for estimating the volume of the islets in small animals have also been described by Mount<sup>28</sup> and by Haist *et al.*<sup>29</sup> both involving pressing the pancreas between glass plates (the latter authors also using neutral red injection) then projecting with a micro-projector, tracing the islets and calculating the volume and weight of the islets in relation to the whole pancreas. These methods are only subject to the same criticisms that apply to all the islets especially the small ones. The work of Haist *et al.* is discussed in Chapter

Susman<sup>20</sup> applied a somewhat similar method to paraffin sections of human pancreas (see Chapter 3)

Thompson *et al.*,<sup>21</sup> from a study of the frequency distribution of the volume of the islands in man, monkey, and dog hypothesized that no new islands are formed after prenatal life

Until the work of Bensley<sup>11</sup> the distinguishing characteristics of the island cells were chiefly negative the most important being the absence of zymogen granules. Specific granules had been demonstrated in the island cells by Laguesse<sup>22</sup> in 1896 and the presence of two different types of cells having been detected by Schulze<sup>23</sup> and Diamare<sup>24</sup> the path for further study was opened by Lane<sup>25</sup> who devised methods for distinguishing the two types of granules from one another and also from the zymogen granules

Two types of island cells are commonly recognized the  $\alpha$  cells and the  $\beta$  cells. Unstained the granules of the two different types of cells cannot be distinguished from one another. The granules of the  $\alpha$  cells are stained violet by safranine and violet following Lane's chrome-sublimate fixation and the  $\beta$  granules are stained red. With the same fixation and acid fuchsin after the neutral gentian stain the  $\alpha$  cells are red and the  $\beta$  cells violet. With the modification of Mallory's aniline blue which we have found very useful, the  $\alpha$  granules are stained red and the  $\beta$  granules are stained bluish purple. (See Plate I) This same result is obtained by Ullrich's stain. With Gomori's chrome-alum hematoxylin stain the  $\beta$  cells are gray-blue with various

island cells were not preserved or were not stained the protoplasm was finely alveolar and that as post-mortem degeneration progressed a coarser alveolar structure appeared. This has an important bearing on the question of hydropic degeneration.

In addition to the presence of specific granules the types of island cells can be distinguished by nuclear characteristics. Both types of island cells lack the prominent nucleus of the acinar cell. The nuclei of the  $\alpha$  cells are poor in chromatin and are usually oval in outline. The nuclei of the  $\beta$  cells are usually spherical though sometimes slightly oval and contain relatively large granules of chromatin. In addition to these two types of cells cells are found without granules whose nuclei closely resemble those of the  $\alpha$  cells. The  $\beta$  cells form the greater bulk of the island. Gomori<sup>26</sup> one of the leading students of the pancreas states that in normal human islands about 60 to 90 per cent of all cells are  $\beta$  cells, 2 to 5 per cent D cells and the rest  $\alpha$  cells.

\* Lane<sup>25</sup> who gave the first definitive description of these cells called the cells which he thought were fixed by alcohol "A" cells and those fixed by his aqueous chrome-sublimate solution "B" cells. Bensley<sup>11</sup> writing shortly afterward called them A and B. There seems to be a tendency in the recent European literature to use the latter nomenclature. However since it is customary in this country to speak of alpha and beta cells we have chosen to use the Greek letters.

In the guinea pig Bensley<sup>11</sup> found no definite arrangement of the  $\alpha$  and  $\beta$  cells in the islands. They also occur without definite arrangement in islands of the human pancreas although the  $\alpha$  cells appear a little more frequently at the periphery of the islands.

Tschassownikow<sup>12</sup> suggested that the  $\alpha$  cells were intermediate between the acinar cells and the  $\beta$  cells. However Bensley<sup>11</sup> has shown that they occur in large numbers in the interstitial islands which are developed directly from the ducts; they are present in the interior of the island as well as at its periphery; they are present in the acini of newborn guinea pig without  $\beta$  cells and they disappear from the acini within the first week of life without any increase in the number of  $\beta$  cells. Bensley concludes that the two types are independent and that each develops from an undifferentiated duct cell. Bloom<sup>13</sup> found a type of cell which one of us (S. W.) described in the first edition of this book (page 36) to be so constantly present that he described it as a third type of island cell, the D cell, which is about as frequent as the  $\alpha$  cell. The D cell granules stain light blue after formaldehyde-chrome-sublimation fixation and the Mallory azan stain.<sup>14</sup>

These observations were confirmed by Gomori<sup>1</sup> who stated that he found definite transition forms between the D and  $\alpha$  cells. In three papers<sup>15, 16, 17</sup> this author has given an admirable summary of the application of specific staining methods to the pancreas of numerous animals as well as man. The D cells are not demonstrable with the neutral stains of Lane, Bensley, and Bowie, nor are they shown by Gomori's chrome-rum-hematoxylin-phloxine stain which is however excellent for distinguishing the two main cell types. They are shown by his new aldehyde-fuchsin-trichrome method.

In addition to the granules the island cell contains mitochondria which appear as delicate filaments and fine granules. They are scattered throughout the cell in marked contrast to their concentration at the base of the acinar cell. Among the granules of the island cells are lipid droplets and also clear cisterns which may be identified by appropriate staining as Golgi apparatus.

O'Leary<sup>18</sup> has followed *in vivo* the formation of vacuoles within the island cells and the migration of these vacuoles toward the capillary end of the cell where they gradually decrease in size. It is possible that these may be secretory vacuoles. It may be that the appearance of hydropic degeneration is due to the formation of large numbers of the vacuoles and in view of Toreson's work cited in the next chapter the possibility of their containing glycogen must be considered.

Saguchi<sup>19</sup> has described in the frog's pancreas five types of island cells which have transitional forms between certain of them. In human tissue we have been unable to parallel Saguchi's types.

Throughout 41 representative mammalian species Thomas<sup>4</sup> has found  $\alpha$ ,  $\beta$ , and D cells. The C cell occurs only in the guinea pig. In this range of species the size and organization of the islands differ widely. Differential

# PLATE I



NORMAL PANCREAS

acinar cells contain orange-red granules.  $\beta$  cells light blue granules. D cells dark blue granules. The duct epithelium stains a very light blue. The acinar cell granules are purple. By a slight modification of the zymogen granules can be shown in light as well as in dark. It is due to the presence of fat. In comparison with Figure 1 (1) shows that the amount of fat demonstrated here is not excessive. No fat at all at age fifty-seven years. Not histologic. Modified Mallory's and cell stain.



staining is always necessary to identify the cell types as size of both cells and granules varies except within a few small groups of minims.

Gomori<sup>2</sup> points out the possibility of confusion when the so-called Minimowski cells are present. These are probably degeneration products of acinar cells. They contain acidophilic granules finer than zymogen granules but coarser than  $\alpha$  granules. Certain stains permit their differentiation.<sup>40</sup> They are perhaps identical with the onkocytes mentioned above.

Mitotic figures are occasionally encountered in the island cells of normal pancreases. The occasional presence of mitotic figures in the  $\beta$  cells may be regarded as evidence against the transformation of  $\alpha$  into  $\beta$  cells. (See below.)

Well or even poorly circumscribed islands do not make up all the insular tissue. With the aid of special stains very small clusters of otherwise ill-defined cells are distinguishable among acini and duct cells.

Masson<sup>41</sup> demonstrated no argentaffin granules in island cells and O'Leary and Womack<sup>42</sup> point out the importance of this in differentiating island adenomas from intestinal carcinoids.

While a true argentaffin reaction (in the sense of a substance within the cell which itself has the power of reducing silver without the addition of any other reducer<sup>43</sup>) is not present in the islets, silver impregnation methods may be effectively applied to the islets. This was first shown by Piazza<sup>44</sup> later by Lasowski,<sup>45</sup> Nagelschmidt<sup>46</sup> and Ferner.<sup>1</sup> The last-named author has carried out extensive studies with the Gros-Schultze method and on the basis of these has built up a theory regarding the relation between the two main cell types. The essence of Ferner's argument is that the cells which are impregnated by the Gros-Schultze method including the  $\alpha$  cells and various cells scattered among the acini singly or in groups in the connective tissue and in the walls of the ducts represent immature (unreifen) endocrine elements which are capable of forming islet tissue i.e.  $\beta$  cells. In fetal life these silver cells are seen in small buds projecting from the ends of the growing glandular formations and as peg-like excrescences from the ducts. Eventually the early islands round off and develop a core of nonimpregnated central  $\beta$  cells around which the silver cells are still arranged in layers. In the newborn the silver cells and the islets are much more numerous than in the adult but according to Ferner this makes no difference in physiological terms since the silver cells are regarded as nonfunctional \* resting forms which act merely as precursors of the  $\beta$  cells. As evidence in support of this argument he offers an experiment in which long-continued administration of insulin to guinea pigs resulted in an increase in silver cells, reflecting a reversion to the resting stage because of lack of demand for insulin production. He also suggests that the same thing may occur in man citing the case of a young executed woman.

\* More recently Ferner seems to have modified this view somewhat and to have accepted the hypothesis that the  $\alpha$  cells may produce a hormone (see below).





staining is always necessary to identify the cell types as size of both cells and granules varies except within a few small groups of islets.

Gomori<sup>3</sup> points out the possibility of confusion when the so-called Mikowski cells are present. These are probably degeneration products of acinar cells. They contain acidophilic granules finer than zymogen granules but coarser than  $\alpha$  granules. Certain stains permit their differentiation.<sup>40</sup> They are perhaps identical with the monocytes mentioned above.

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(other reducer<sup>43</sup>) is not present in the islets. Silver impregnation methods may be effectively applied to the islets. This was first shown by Pizzani<sup>44</sup> later by Lasowsky,<sup>45</sup> Nagelschmidt<sup>46</sup> and Ferner.<sup>7</sup> The last named author has carried out extensive studies with the Gros-Schultze method and on the basis of these has built up a theory regarding the relation between the two main cell types. The essence of Ferner's argument is that the cells which are impregnated by the Gros-Schultze method including the  $\alpha$  cells and various cells scattered among the acini singly or in groups in the connective tissue and in the walls of the ducts represent immature (unreifen) endocrine elements which are capable of forming islet tissue i.e.  $\beta$  cells. In fetal life these silver cells are seen in small buds projecting from the ends of the growing glandular formations and as peg-like excrescences from the ducts. Eventually the early islets round off and develop a core of nonimpregnated central  $\beta$  cells around which the silver cells are still arranged in layers. In the newborn the silver cells and the islets are much more numerous than in the adult but according to Ferner this makes no difference in physiological terms since the silver cells are regarded as nonfunctional \* resting forms which act merely as precursors of the  $\beta$  cells. As evidence in support of this argument he offers an experiment in which long-continued administration of insulin to guinea pigs resulted in an increase in silver cells reflecting a reversion to the resting stage because of lack of demand for insulin production. He also suggests that the same thing may occur in man citing the case of a young executed woman.

\* More recently Ferner seems to have modified this view somewhat and accepted the hypothesis that the  $\alpha$  cells may produce a hormone (see below).

who had apparently been starved in prison and in whom he felt he could demonstrate an increase in silver cells.

Ferner seems uncertain as to the relation of the silver cells to the clear cells of Leyter.<sup>41</sup> Apparently he feels that the clear cells may precede the silver phase. He rejects the idea of Frisvolder<sup>42</sup> that the silver cells of the pancreas are essentially identical with the preentero-chromaffin cells of the intestine and points out that there is a fundamental histochemical difference in that the enterochromaffin cells have within their cytoplasm the property of reducing silver salts whereas in the pancreas the silver cells require the application of an added reducer (such as formalin) to bring out the reaction. Strictly speaking the term argentaffin should be restricted to the former type of cell i.e. that which is impregnated by the Masson Fontana method without the addition of a reducing agent. (See Gomori<sup>43</sup>)

Presumably the silver cells of Lerner are identical with the muddy (truben) cells which Neubert<sup>4</sup> describes as progenitors of the islets in the fetus.

Ferner also demonstrated that the  $\alpha$  cells would give a positive vitamin C reaction by the method of Giroud and Leblond. The significance of this is obscure. Administration of vitamin C to a guinea pig did not appear to cause an increase in these cells. Whether this finding is related to the supposed hyperglycemic hormone of the  $\alpha$  cells or to a corbic acid diabetes is unknown.

Ferner's conclusions are accepted apparently completely and without question by Hess.<sup>40</sup> Others however have shown more skepticism. Creutzfeldt<sup>5</sup> and Bargmann and Creutzfeldt<sup>44</sup> have questioned the ability of  $\alpha$  cells to turn into  $\beta$  cells. They point out that if this were true one would expect to find evidence of it in alloxan diabetes but on the contrary in this condition the  $\beta$  cells have been seen to multiply by mitotic division.<sup>45</sup> Terbruggen<sup>46, 47</sup> notes that mitotic figures may also be observed in the  $\beta$  cells of adult humans (see also Warren and Root<sup>48</sup>) and that in some islet adenomas the  $\beta$  cells appear to spring from nongranular precursors. He prefers to regard the  $\alpha$  cell as either a resting phase of the islet cell or as a special cell system. Finally the wise remark of Gomori<sup>5</sup> may be cited that if one is to postulate transformation of one of these cell types into another one should be able to find transition forms containing both types of granules in the cytoplasm. Using this criterion Gomori<sup>49</sup> was able to find plenty of evidence for transition forms between D cells and  $\alpha$  cells but none for transition between  $\alpha$  and  $\beta$  cells.

Recently Ferner and Stoeckenius<sup>57</sup> have supplemented Ferner's earlier studies with an investigation of the developing fetal islands using chiefly the chrome-alum hematoxylin and phloxine method of Gomori. They describe a stage which they call the mantle island where there is a peripheral mantle of  $\alpha$  cells surrounding a central nucleus of  $\beta$  cells with an intervening layer of cells with a homogeneous nongranular rose-colored

cytoplasm. The latter cells they maintain represent transition forms between  $\alpha$  and  $\beta$  cells. They also describe the  $\alpha$  and  $\beta$  cell as showing nuclear differences which are very striking in the guinea pig, much less so in man. Lerner has also written a general review<sup>20</sup> with particular emphasis on the hypothetical glucagon or H.G. factor, supposedly produced by the  $\alpha$  cells, thus apparently modifying his earlier view noted above that the  $\alpha$  cells are nonfunctional immature precursors of the  $\beta$  cells.

**Transition Theory** The possibility of transition between island and acinar cell is obviously of great importance particularly in the diabetic pancreas. However there is now little but historical interest in the question thanks particularly to the masterly studies of Bensley. The evidence brought forward by Laguesse<sup>21</sup> and others to support the transition theory faded before the demonstration of specific granules in the island cells. Suguchi<sup>2</sup> held that the granules of the  $\alpha$  and  $\beta$  cells do not take active part in the specific secretion of the island cells but that the  $\delta$  cells with their lipid corpuscles and urano argentophile apparatus are the most important. He further believed<sup>22</sup> the acinar cell may possess an internal secretion and that the acinar cell may change into an island cell with changes not only of the cell body and nucleus but of the intracytoplasmic structures as well. Much of his work was based on the frog and has not been confirmed for other species.

Otani<sup>1</sup> has also endeavored to revive the old controversy basing his evidence for transition between the islands and the acini not so much upon the specific character of the island cells as on the arrangement of the cells and the relations of the islands to the acini laying special stress on the absence or presence of the capsule.

The existence or nonexistence of a connective-tissue capsule about the island has been regarded as of importance by many in determining the entity of the island-cell group. The Kapsul Frage has been hotly contested. However the frequent occurrence of an imperfect connective-tissue capsule is undeniable, also its usually incomplete character. A complete connective-tissue capsule is usually the first sign of a developing fibrosis.

Hirtroft<sup>4</sup> notes the frequency with which reduplication of the capillary basement membrane may be found in the diabetic pancreas if connective tissue stains are applied.

Otani<sup>1</sup> recognizes three types of island structure:

- 1 Most common islands connected with surrounding acini
- 2 Islands connected with ducts
- 3 Least frequent islands strictly separated from the surrounding structures

In the same pancreas he finds wide variations in size, shape and relation of the islands. In addition he believes<sup>4</sup> that acinar cells are able to change into island cells and that the process of hypertrophy of the islands of

Langerhans in the adult pancreas is mostly due to transformation or hyperplasia of acinar and centro-acinar cells. He does not consider the islet as constituting an independent tissue. He also concludes that new formation of the islands of the adult pancreas may occur by transformation and hyperplasia of acinar, centro-acinar or duct cells. The striking experiments of Ukai<sup>13</sup> have emphasized the independence of the island tissue from the acini and have also shown that the insular epithelium is apparently possessed of greater resistance to injury, if not greater powers of regeneration than is the case with the acinar cells.

Recent authors (e.g. Gomori<sup>2</sup>, Ferner<sup>7</sup>, Hard<sup>8</sup>, Bargmann<sup>4</sup>) seem to place little emphasis on the possibility of transformation of acinar to islet cells at least in the adult. Neubert<sup>6</sup> demonstrated the origin of islets from the end pieces of the glands in the fetus. Hughes<sup>5</sup> apparently believes that he has demonstrated acino-insular transformation in the rat by graded doses of alloxan after which the smaller, more sensitive  $\beta$  cells are destroyed and replaced from the periphery by large  $\beta$  cells from the acini, thus pushing the  $\alpha$  cells toward the center of the islet and reversing the usual arrangement. Here again one may apply Gomori's criterion and attempt to find cells containing both zymogen and islet granules. Judged by this standard which of course is not necessarily a valid one, most of the reported work upholding acino-insular transformation must be questioned.

**Blood Supply**—One of the most striking of the grosser proofs of the autonomy of the islands and one of the most important facts from the standpoint of their function is their peculiar blood supply. Whereas the acinar tissue receives a liberal blood supply from the lobular arteriole with its interacinar capillaries, the islands receive an extraordinarily rich supply and have wide anastomosing sinusoids in almost glomerular arrangement coursing through them. This sinusoidal net is usually fed from one or more arterioles, but sometimes is in direct continuity with the interacinar capillaries. A detailed study of the pattern of the insular circulation is given by Beck and Berg<sup>10</sup> and Beck and Peterson<sup>11</sup> have demonstrated a particularly rapid blood flow through the islands. The capillaries of the islands are thin walled and usually without fibroblastic support. In a case of marked chronic passive congestion shown to one of us (S.W.) by Dr S. B. Wolbach, this rich supply is even better shown than in injection preparations.

**Nerve Supply**—Between the island cells are the terminations of numerous nerve fibers which form a network around the islands<sup>12</sup>. Lenard<sup>14</sup> detected numerous nerve fibers entering the islands with the blood vessels and distributed along them and between cells. These fibers he felt to be different in number and arrangement from those supplying the acini. Similar conclusions were reached by Ritter<sup>15</sup>. Rarely small ganglia are encountered. Pacinian corpuscles are present in the human pancreas and are very common in that of the cat.

Simard<sup>16</sup> has made a careful study of the so-called neuro-insular com-

plexes in various mammals including man. These bodies consist of an intimate association of nervous and epithelial elements and are situated in the inter- and intralobular septa. They differ in the relative number of epithelial and ganglionic components. The epithelial cells are islet cells, both  $\alpha$  and  $\beta$  types. Simard seems to think that there is some evidence for renewal of the complexes by acino-insular transformation. He regards them as metasympathetic paraganglia and suggests that they might be chemoreceptors. He apparently believes also that the epithelial cells may be transformed into ganglion cells.

Excellent general reviews of the cytology of the pancreas have been written by Opie<sup>22</sup> Gomori<sup>3</sup> and Burgmann.<sup>6</sup>

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this lesion was that of a girl aged seventeen years whose diabetes began two years before death.

One of the arguments brought forward in favor of the epithelial origin for hyaline in the islands is that with Mallory's and blue stain certain of the island cells assumed to be those which later will be replaced by hyaline stain intensely blue and that later masses of hyaline appear in them which ultimately fuse forming large masses about the capillaries. One of these cells is shown in Figure 3. In many cases of diabetes examined

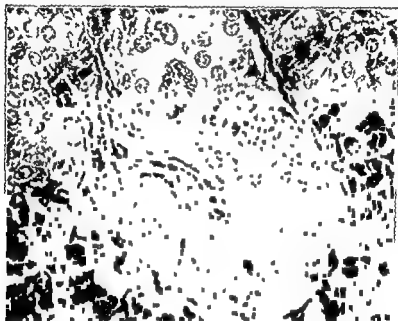


FIG. 3. Island showing large degenerating cell. No. 3015. Female aged forty-eight years. Duration of diabetes three years. Mallory's and blue stain.  $\times 560$ .

in this laboratory bluish staining cells similar to those described can be seen provided the tissue is fresh and the fixation satisfactory. As a rule they are somewhat irregularly distributed through the substance of the islands. At other points small masses of hyaline can be seen. However similar blue-stained cells can be found in the islands of nondiabetic individuals and in islands which show no trace of hyalinization. The blue-stained cells occur scattered throughout the island and do not show the definite relationship to the blood vessels that the hyaline does. These cells have been named B cells by Bloom.<sup>2</sup>

Although admittedly unreliable the staining reactions of this hyaline throw some further light on its nature. In the first place out of 31 cases of hyalinization of the islands suitably fixed following the suggestion of Dr. F. B. Mallory, one of us (S. W.) was able to demonstrate a positive

## PLATE II



HYPERTROPHIC AND OPACIFICATION

Mild to moderate hyperplasia of alveolar epithelium  
 with increased vascularity. The alveolar spaces are filled with  
 large numbers of cells. Magnification  $\times 100$



amyloid reaction with methyl violet or iodine green in 14. Recently several investigators have given considerable attention to this point. Gellerstedt,<sup>4</sup> chiefly on the basis of studies with the methyl violet reaction, concluded that the hyaline is actually amyloid and that the condition should be called 'insular (para)amyloidosis'. He holds that the peculiar localization in the islets is to be regarded as one manifestation of so called senile amyloid deposition which he looks upon as a common phenomenon, stating that careful search will reveal amyloid in the tissues of around 45 per cent of males over 50 years of age. Van Beek<sup>7</sup> made similar observations. Arey<sup>8</sup> and Ahronheim<sup>9</sup> also hold that the hyaline found in the islands of diabetics is actually "or" "in all probability" amyloid basing their conclusions on the usual staining reactions. The fact that the hyaline is deposited in contact with the walls of blood vessels in the islands as noted in previous editions of this book, is emphasized by Arey,<sup>8</sup> who found hyaline in the pancreases of 16.6 per cent of nondiabetics and 71.7 per cent of diabetics over fifty years of age. He states that the hyaline of the islets may not be identical with the amyloid in the liver and spleen in cases of generalized amyloidosis since it gives a somewhat lighter stain than the usual amyloid with methyl violet, hematoxylin and eosin and the Mallory-Heidenhain method. Ahronheim<sup>9</sup> found the hyaline more abundant in the younger obese group of diabetics. He obtained positive reactions in 67 of 105 diabetics and in 5 of 50 nondiabetics of similar age. Many of his patients in both categories had hypertension. He emphasized that amyloidosis of the islands is an isolated feature not usually associated with amyloid in other tissues.

The entire concept of atypical amyloid or paramyloid is an exceedingly vague one.<sup>10-14</sup> For instance Bauer and Kuzma<sup>12</sup> state: "The term paramyloid signifies the atypical distribution and variable staining reaction of a hyalin like material. Such a definition obviously allows for the inclusion of a variety of substances of different chemical nature."

King<sup>10</sup> points out that it is illogical to classify amyloid as "primary" and "secondary" and prefers the term "typical" and "atypical" basing his classification on anatomic distribution of the deposit. He also describes a simple silver stain. Amyloid may well be a protein of variable composition the various strains reacting with different prosthetic groups.<sup>15, 17</sup>

Perhaps as suggested by one of us (S. W.<sup>16</sup>) atypical amyloidosis is a reflection of dysfunction of fibroblasts. Jones and Irazek<sup>17</sup> found an intimate relation between reticulum fibers and amyloid deposits. They note also that amyloid stains red with leukofuchsin and suggest that it may represent a mucopolysaccharide combined with a variety of different proteins (including those of reticulum as well as those involved in antigen-antibody reactions). (See Chapter 28 for further discussion of this concept.)

Certainly amyloid is frequently associated with hyperglobulinemia (Jellum<sup>18</sup>) but to assume that it is simply excess globulin deposited from the blood stream would seem unwarranted in the present state of our knowl-

## THE PANCREAS IN DIABETES MELLITUS

edge. Indeed it seems quite arbitrary to include as Teilum<sup>11</sup> does cases in which the usual methyl violet and Congo red reactions are negative. A classification without histochemical basis would appear to be open to question.

Since the chemical nature of atypical amyloid or paramyloid if it be an entity is unknown<sup>12, 13</sup> and the substance is defined by certain non-specific tinctorial reactions and since these reactions are found inconstantly in hyalinized islands of Langerhans it would seem wise to withhold judgment. Labeling the hyaline material amyloid does not solve any problems but rather raises new ones and until its chemical nature is better defined it is perhaps preferable to refer to it by the usual term of hyaline. Indeed it might prove more fruitful to emphasize the polysaccharide component (See Chapter 28.)

The hyaline ordinarily gives the following staining reactions:

- Mallory's aniline blue—translucent blue
- Phosphotungstic acid hematoxylin—brown
- Phosphomolybdic acid hematoxylin—deep blue
- Eosin methylene blue—pink
- Masson's iron hematoxylin light green—bright green
- Iron hematoxylin in Gieson—yellowish
- Best's carmine stain—pink to old rose
- Periodic acid-Schiff reagent—pale pink

The staining reactions obtained from hyaline about the arterioles of the spleen from several cases which also showed hyalinization of the island of Langerhans were identical. Occasionally a slight fibrillar structure can be demonstrated in the hyaline.

Study by the serial section method shows that the masses of hyaline are always in contact with the walls of the vessels of the islands and always intercellular. Masses that are apparently isolated can always be traced in the course of one or more sections 3 microns thick to the perivascular deposit of which they are a portion.

Should hyaline be the result of cellular degeneration secondary to diabetes it ought never to be present in the absence of diabetes. However cases have been reported by Cecil<sup>14</sup>, Wright<sup>15</sup>, Arey<sup>16</sup>, Ahronheim<sup>17</sup> and others where hyalinization of the islands was present in nondiabetics. It is true that in a few of these cases objections can be raised on the ground that the individual might be in reality diabetic but owing to terminal malnutrition failed to show signs of diabetes just as he might fail to show these signs under rigid dietary treatment. But not all these cases can be thus disposed of.

Moreover of 4 cases in our series where fairly complete clinical data have been available to rule out the occurrence of diabetes mellitus, hyaline has been present in the islands to a moderate degree in 3 cases and to a marked degree in one. Studies of deposition of hyaline in island cell adenomas<sup>18</sup>

where hypoglycemia rather than diabetes exists further confirm this viewpoint. Meissner<sup>11</sup> has found hyaline quite constantly in functional adenomas but not in the nonfunctional ones (Chapter 22) and suggests that it may be related to the secretion of insulin rather than representing degeneration.

Hyaline degeneration is most common in older diabetics, as has already been said, and in mild diabetics. In our material 6 per cent of the cases

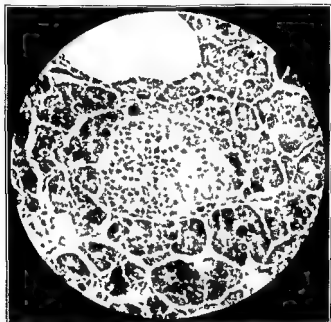


Fig. 4.—Early hyalinization of islet of Langerhans. Male, age thirty-six years. Duration of diabetes fifteen years. Isonitric methanolic fixation.  $\times 170$ .

through forty years of age showed hyaline, as against 15 per cent of those over forty years of age. In carefully studied cases definitely known to have had diabetes over ten years 50 per cent showed hyalinization of the islands of Langerhans.

A striking feature is the unequal involvement of the islands in the same pancreas, some being converted to completely hyalinized masses with only a few fibroblasts and capillary endothelial cells present while others may be completely spared. Still others show varying degrees of transition between these two extremes. This seems to point to either striking inequalities of involvement or to the replacement of destroyed islands by new ones.

Frequently the insular epithelium does not tamely submit to the hyaline invasion but as the hyaline separates the cells from their blood supply and

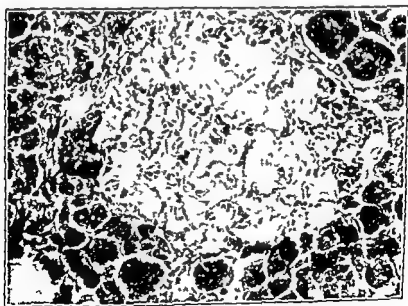
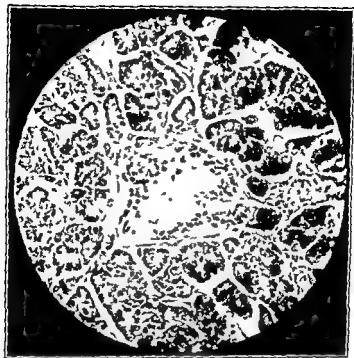


FIG. 6. Mark II valuation of liver of la geria. DA27-9. Fetal aged  
 sixty years. Duration of diabetes five years. Note the few small dark spots  
 at right hand margin of slide. Mallory's blue stain.

finally forces them out of existence growth occurs at the periphery of the island particularly if adjacent to fat or fibrous tissue into which expansion can take place more readily than into the scar tissue. However encroachment on these newly formed cells occurs and finally results in the formation of very large hyaline masses far larger than the original island. The end stage of such a process is shown in Figure 7.



Particular

time

as tumor at right of figure persists  
years. Duration of metastasis  
(normal island) Mallory's aniline

CRIMINAL

13  
974



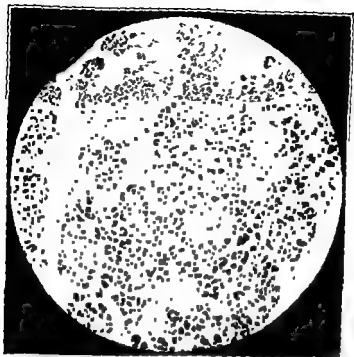


FIG. 8.—Pancreas showing large numbers of hyaline islands. U24-1. Female aged forty-nine years. Duration of diabetes fifteen years.

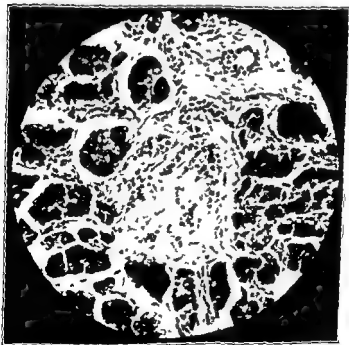


FIG. 9.—Extreme hyalinization of island of Langerhans. Note blood cells in still patent capillaries. M971. Male, aged sixty-six years. Diabetes of fourteen years' duration. Phosphotungstic acid hematoxylin stain.  $\times 170$ .

Rarely the hyaline becomes the seat of calcium deposition so that the pancreas may be studded with minute calcified nodules. One such case is described by Mallory<sup>4</sup> and another by Gibb and Logan.<sup>10</sup> Foscadero<sup>11</sup> states that calcification is extremely rare.

The most remarkable case of calcification of the islands is that of Fischer.<sup>12</sup> The patient, an eighteen year-old boy, died in coma after typical juvenile diabetes. The pancreas weighed 100 grams, was somewhat firm and its cut surface revealed many minute white spots. These proved to be calcified hyalinized islands. Many of the islands were large and they were very numerous. Fibrosis as well as hyalinization was present. While the pancreas was somewhat fibrotic, the acini were not damaged.

Hyalinization of the islands of Langerhans in light of the foregoing evidence may be considered as due either to amyloid (paramyloid) or to production of intercellular substance by fibroblasts and possibly by endothelial cells. When the process becomes sufficiently marked to destroy a number of the epithelial cells or to separate them from the blood stream by a practically impermeable membrane diabetes will develop. It is of no slight interest that this pathological product usually appears in the later years of life about the time that marked hyaline thickening of the small arterioles is prone to occur. The reason for the specific involvement of the islands rather than the acinar tissue is difficult to interpret although the rich vascularity of the islands may provide a clue.

TABLE 5.—LESIONS IN THE ISLANDS OF LANGERHANS IN 811 DIABETIC PANCREASES

Age	Case	Normal	S+	S++	S+++	H+	H++	H+++	Hypertrophy	Hyd. opic change	Pyknotic nuclei	Hem. rhagds	Ad. ism	Lymphocyte infil.
0-10	17													
11-20	15	1*												
1-30	41	00												
11-40	24	1h	2	6										
41-50	85	30	11	10	1	4	11	11						
51-60	106	64	27	1*	6	0	11	6	15	6	4			
61-70	154	00	00	10	4	30	50	50	23	7	4			2
71-80	111	42	00	0	4	00	15	7*	11	5	4	1	1	1
81+	14	4		1	0		1	4	1					
.	4	2		1	1									
Total	811	271	165	63	14	90	114	173	63	57	40	3	6	11

S = fibrosis or sclerosis H = hyaline

Totals apparently do not check because of some constant of lesions.

Through the courtesy of Dr. W. L. Fraumiller, one of us (S. W.) was able to examine the pancreas of a diabetic infant of 5 months of age. It showed a remarkable picture of poly-insulariscentric islands in many islands, together with some lymphocytes and fibrosis of at least one island.

Von Meyenburg<sup>1</sup> calls the picture of lymphocytic infiltration "insulitis" and attributes it to overstrain. One wonders about the possible presence of unrecognized mumps in some of these cases.

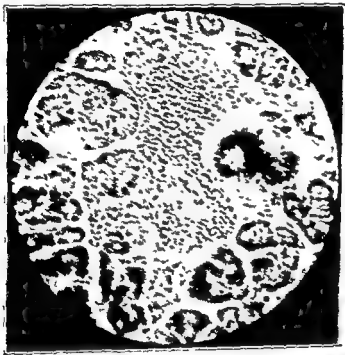


FIG. 11. Marked lymphocytic infiltration and islet fibrosis reducing island of Langerhans. (Mull. Med. and a review of the literature of diabetes eleven months of age.) (Mull. Med. and a review of the literature of diabetes eleven months of age.)

**Histologic Changes.** One of the diabetic lesions was a defect of overproduction from the pathological standpoint is histologic change, described by Woodworth<sup>2</sup> and further emphasized by Allen.<sup>3</sup> On the basis of experimental work a list of "islet changes" Allen<sup>3</sup> recorded as follows:

- (A) In progressive and chronic diabetes.
- (B) In acute diabetes.
- (C) It adds to the evidence of clinical diabetes.
- (D) It explains the pathologic changes in the pancreas.

(E) From a broader physiological standpoint it offers the only proved example of anatomical breakdown of cells due to overstimulation of an internal secretory function

There has been no question from the early experiments of Allen<sup>21</sup> and of Homans<sup>1</sup> to the present time that hydropic change is an important lesion in experimental diabetes (See Chapter 2c) There are so many factors to be considered in human diabetic material however that its interpretation is more questionable there

The striking feature of hydropic change is that it appears to be not the result of injury but of excessive functional strain Most of the intracellular reactions to injury short of necrosis are represented by the liberation of fatty or albuminous and related substances from the protoplasm giving the pictures commonly described as fatty degeneration albuminous de-

until the cytoplasm of the cell is apparently replaced by a watery fluid leaving the nucleus alone in the embrace of the distended cell membrane Apparently this change is reversible in its slighter degrees but once fully established leads to the atrophy and disappearance of the cell involved If the work of Torsen (see next section below) is confirmed and it is found that the hydropic appearance of the cell is actually due to glycogen then much of the difficulty in the concept of reversibility of the lesion will disappear

It is striking that this response to functional overstrain is unique among the endocrine glands and indeed among all the various organs Perhaps the simplest physiological analogy to the pancreas in diabetes would be the thyroid in cases of myxedema yet in the thyroid cells no such anatomical change develops

It is rather difficult to explain all cases of hydropic change on the basis of functional overstrain of the  $\beta$  cells In going over the earlier series of diabetic pancreases one of us (S W) was struck by the frequent occurrence of appearances suggesting hydropic change in many of the island cells of the pancreases removed over three hours post mortem and the comparative rarity of this finding in pancreases removed one hour or less post mortem Allen<sup>22</sup> believes that hydropic degeneration is demonstrable in the human pancreas whenever diabetic symptoms have been sufficiently intense and prolonged but is ordinarily missed in the mildest cases He also emphasized the importance of fresh tissue for its demonstration However as pointed out by Connor<sup>23</sup> it is difficult to see how a cell with cytoplasm practically replaced by vacuolization or by one large vacuole could through post mortem change acquire normal-appearing cytoplasm

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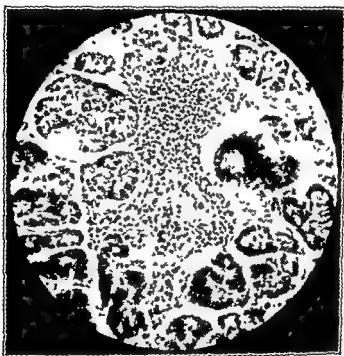


FIG. 11.—Marked lymphocytic infiltration and slight fibrosis replacing island of Langerhans. M942. Male aged nineteen years. Duration of diabetes eleven months. Four methylene blue stain.  $\times 170$ .

**Hydropic Change**—One of the diabetic lesions most difficult of comprehension from the pathological standpoint is hydropic change, described by Weichselbaum,<sup>23</sup> and further emphasized by Allen.<sup>24</sup> On the basis of experimental work and clinical observations Allen<sup>24</sup> regarded this lesion as of much importance on the following grounds:

"(A) Its presence affords a positive microscopic diagnosis of active diabetes.

"(B) It completes the proof of the island theory of diabetes.

"(C) It adds to the evidence of the essential identity of experimental and clinical diabetes.

"(D) It explains the permanent lowering of assimilation in diabetes consequent upon excessive diets.

(E) From a broader physiological standpoint it offers the only proved example of anatomical breakdown of cells due to overstimulation of an internal secretory function.

There has been no question from the early experiments of Allen<sup>21</sup> and of Homans<sup>22</sup> to the present time that hydropic change is an important lesion in experimental diabetes. (See Chapter 25.) There are so many factors to be considered in human diabetic material, however, that its interpretation is more questionable there.

The striking feature of hydropic change is that it appears to be not the result of injury but of excessive functional strain. Most of the intracellular reactions to injury, short of necrosis, are represented by the liberation of fatty or albuminous and related substances from the protoplasm, giving the pictures commonly described as fatty degeneration, albuminous degeneration or cloudy swelling, colloid degeneration and so forth.

The typical hydropic change is totally different from these. The specific granules disappear and are replaced by vacuoles, which grow and coalesce until the cytoplasm of the cell is apparently replaced by a watery fluid leaving the nucleus alone in the embrace of the distended cell membrane. Apparently this change is reversible in its slighter degrees, but once fully established, leads to the atrophy and disappearance of the cell involved. If the work of Torsion (see next section, below) is confirmed and it is found that the hydropic appearance of the cell is actually due to glycogen, then much of the difficulty in the concept of reversibility of the lesion will disappear.

It is striking that this response to functional overstrain is unique among the endocrine glands and, indeed, among all the various organs. Perhaps the simplest physiological analogy to the pancreas in diabetes would be the thyroid in cases of myxedema; yet in the thyroid cells no such anatomical change develops.

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Allen<sup>25</sup> also considers that the pathology of diabetes indicates "the diabetic pancreas represents in most cases a burned-out configuration and the

chief or sole cause of aggravation of the condition lies in hydroptic degeneration of the islands

The relation to duration of the diabetes is somewhat uncertain but the most striking cases of hydropic change which we have seen have been in fulminating cases of diabetes of relatively short duration. Dr John R. Williams give the privilege of studying the pancreas of one case of only three weeks' duration which showed marked hydropic change. Several of the early cases of diabetic children with severe diabetes progressing rapidly to coma showed suggestive changes but post mortem change could not be ruled out.



FIG. 19. Pancreas showing apparent hydropic degeneration. Note extreme vacuolation. This appearance was seen in 86. Four hours post mortem.

IN THE LIVER OF THE PANCREAS

It may be objected that the rarity of hydropic change in this series (only 32 cases) is due to the large number of insulin-treated cases on the theory that insulin by reducing functional strain reduces the frequency of the lesions.

If Allen's statement holds that the chief cause of aggravation of diabetes lies in hydropic degeneration of the islands this is of great importance for the above interpretation can be put on observations from this laboratory.

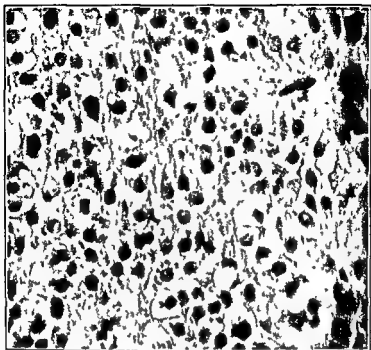
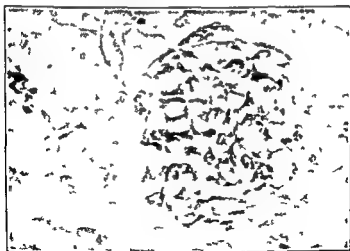


FIG. 13 — So-called hydropic change in islet of Langerhans in rat bit made hyperglycemic with large doses of cortisone. The vacuolated cells were shown to contain glycogen (see Figure 14)  $\times 650$





if we accept functional overstrain as the cause of this change and expect if the appearance is actually due to the presence of glycogen (see below). Assigning to hydropic change such an important role and finding it absent in insulin treated cases the implication is that such cases should not tolerate. However the change is nearly as rare in cases not given insulin (some dying before the Bunting Fri some during it) as in those treated with insulin.

We have seen pancreases from pre insulin cases of diabetes of short duration dying in coma and pancreases from mild diabetics dying from arteriosclerosis or intercurrent infection after twenty five or thirty years of diabetes and neither group showing evidence of hydropic change. The cases of apparent hydropic change were encountered in the control series and the histories of both when carefully studied gave no evidence of diabetes.

In no case of the series reported by Root and Warren<sup>22</sup> was hydropic change positively diagnosed. Conroy<sup>23</sup> before the days of insulin found hydropic change in only 1 of 12 diabetic pancreases exhaustively studied.

While the existence of hydropic change as a clear-cut lesion in experimental animals and human diabetics is unquestionable nevertheless it can be very closely simulated by post mortem change. Such a case is well shown in Fig. 12. Several times one of us (S. W.) has succeeded in producing apparent hydropic change by the simple expedient of leaving a portion of normal pancreas at room temperature for eight or ten hours before fixation. The lapse of many hours between death and autopsy in most of Weichselbaum's cases may have some bearing on the frequency with which he found the lesion.

**Glycogen**—It is possible that the entire concept of hydropic change in the islands of Langerhans may have to be revised in the light of the new work of Toreson<sup>24</sup> which will require extensive study and confirmation.

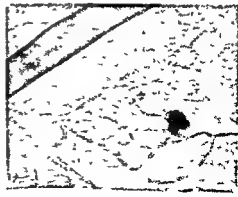
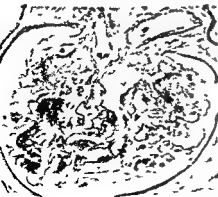
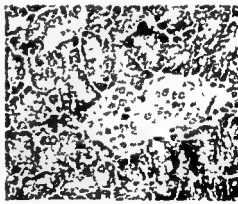
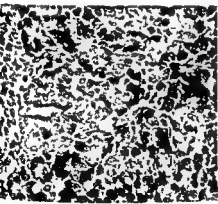
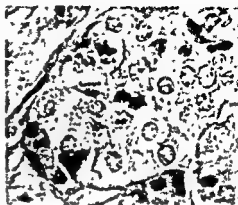
#### LEGEND TO PLATE III

#### METHOD

C. Island of Langerhans of normal rat. Comoros chrome alum hematoxylin and picro-nigrosin stain.

D. Island of Langerhans from rabbit made permanently diabetic with alloxan. It consists entirely of  $\alpha$  cells with the possible exception of three or four  $\beta$  cells.

PLATE III





This investigator has shown that in at least some instances vacuolation of the cytoplasm of the cells of the islands is associated with the deposition of glycogen. His results are particularly striking in experimental animals. Through the courtesy of Dr. Töreson we have had the privilege of examining some of his beautiful preparations showing vacuolated cells of the islands loaded with glycogen in a dog made diabetic with anterior pituitary extract in a rabbit given alloxan and in a rabbit treated with cortisone. The glycogen deposits are massive, are demonstrable by both the Best carmine and periodic acid-Schiff reagent methods and can hardly be attributed to artifact. We have been able to confirm his findings in a rabbit given massive doses of cortisone (Figs. 13 and 14). In Plate III there is illustrated an example of hydropic change apparently due to glycogen as indicated by the failure to stain with the PAS method after treatment with diastase.

In human material the results are less convincing, perhaps due in part to post mortem hydrolysis of the glycogen. In the preparations which Dr. Töreson sent and in some of our own cases fine granules of glycogen can be demonstrated (Plate III) which are removed if the slide is previously subjected to the action of diastase or saliva. However it is not always clear that these granules are associated with vacuolation of the cells. Probably further work will need to be done to establish whether the microscopic appearance which has been called *hydropic degeneration* in the past is actually due to the presence of glycogen or whether glycogen may not be deposited with or without vacuolation in the cells of the islands as a part of the general disturbance of carbohydrate metabolism in diabetes in which case it would be analogous to the similar deposits in the renal tubules, nuclei of liver cells and iris epithelium (see Chapter 5). If Töreson's work is confirmed another point which will require clarification will be the question of why previous investigators who studied hydropic change with great thoroughness (e.g. Allen) did not encounter glycogen. One of us (S.W.) has in the past done many stains for glycogen successfully demonstrating this substance in the duct epithelium but not being impressed with its presence in the islands.

If Töreson's conclusions are indeed correct their theoretical implications will be considerable. The whole question of whether hydropic change does indeed represent injury or functional overstrain of the islands as has always seemed to be true will have to be re-examined. If it does represent damage to the cells the situation is a unique one: there is no evidence as far as we are aware that the long-continued presence of glycogen in the renal epithelium, liver cells or iris epithelium of the eye exerts any injurious effect whatever. Injured cells may become vacuolated due to the presence of fat or fluid but glycogen is usually not found under such conditions.

If one accepts the usual view outlined in the previous section (and in Chapter 25) that hydropic change does indeed represent the earliest visible

evidence of injury to the cells and if one assumes that the presence of glycogen is the explanation for the vacuolation then one is led to wonder why

of  
is

betes as well (see Chapters 25 and 26). An adequate explanation would be provided for the experimental production of hydropic change by massive doses of glucose<sup>27</sup> and for the observations of Gomori<sup>28</sup> who noted vacuolation of the  $\beta$  cells in surgical biopsies of the pancreas from patients who had had large amounts of glucose pre-operatively. The presence of glycogen in the vacuoles would also of course explain quite well the puzzling reversibility of the lesion in the experimental animal and apparently in man.

Much remains to be done in relation to Tolson's fascinating observations. If for example it could be shown that so-called hydropic change is actually related to the level of the blood sugar as is the vacuolation of the renal epithelium many of the puzzling considerations relating to severity and duration of diabetes, effect of insulin and the like discussed above would disappear. Actually hyperglycemia may not be the important factor as is suggested by the work of Duff and Tolson<sup>29</sup> who were able to prevent or reverse the hydropic change in alloxan diabetes of rabbits by giving small doses of insulin even in the presence of sustained hyperglycemia.

It is of interest that Medwedeff<sup>30</sup> described glycogen in the islets in a single case of diabetes in 1930.

**Pyknotic Nuclei**—One appearance in the islands which is interpreted only with considerable difficulty is the presence of pyknotic nuclei and somewhat shrunken cells. Occasionally islands are found closely resembling normal ones except for the presence of extreme pyknosis of the nuclei and acidophilic cytoplasm. This change was encountered in 6 cases. These cases ordinarily show no other lesion in the islands, if indeed this nuclear change may be considered a lesion. One cannot help but feel that it may be either post mortem change or improper fixation that produces these shrunken extremely dense nuclei. However cases autopsied soon after death and showing no evidence of post mortem change do show these pyknotic nuclei in the island cells. Gibb and Logan<sup>31</sup> encountered these islands in 15 cases among 142.

**Necrosis**—Island necrosis of the islands is very rarely found at autopsy. It may occur as a complication of infectious disease but is seen in its striking form as a result of injection of alloxan either in the experimental animal or in the few cases in which the drug has been used in man. The necrosis induced by alloxan is amazing not only for its specificity for the  $\beta$  cells of the islands but also for the fact that the process of cell death goes on in the almost complete absence of any cellular reaction in the form of leukocytes.

**Hypertrophy**—Hypertrophy of the islands is not infrequently encountered. Cecil<sup>11</sup> has pointed out two types of hypertrophy of the islands—one a simple increase in size of the island without change in the character of the individual cells, the other a change in the cells to a columnar type with the nuclei located centrally. Rudimentary islands of this latter type Weichselbaum<sup>12</sup> encountered in 19 out of 151 cases of diabetes and regarded them as representing regeneration of islands from duct epithelium. Cecil agreed that this type of hypertrophy is seen in islands newly formed from duct epithelium. He found 34 cases of hypertrophy and

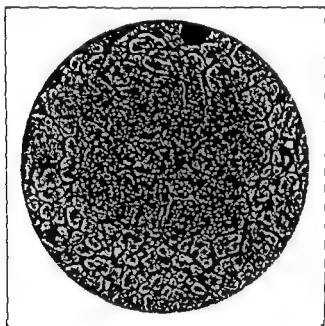


Fig. 15.—Hypertrophy of island of Langerhans. Aorta. Not distinct. I. out. methylene blue stain.  $\times 40$ .

probable regeneration in 100 diabetic autopsies, none in 33 cases of chronic pancreatitis, and only 1 in 17 cases of circumscribed involvement of the pancreas. MacCallum<sup>13</sup> gives an excellent description also.

The two types of hypertrophy are easily distinguished. The coiled snake-like columns of cells in the islands in the columnar type are quite different in appearance from the typical sinusoidal structure, although the close relation of cells and vessels is maintained. The epithelial cells themselves are somewhat changed, tending toward a columnar rather than the usual cuboidal polyhedral shape. Irregular projections among the acini at the periphery of the island are common. This change appears to have no definite correlation with the pathological physiology.

Hypertrophy as well as hyperplasia of the islands is seen commonly in the infants of diabetic mothers (see Chapter 23)

Ogilvie,<sup>45</sup> using the method described previously by him (see Chapter 2) has more recently made a special study of hypertrophy of the islands. He finds enlarged islands particularly in obese persons and ascribes them to a pancreotropic factor of the pituitary. (See page 22 for further references on the volume or total mass of island tissue)

On occasion it may be difficult to distinguish large islands from small adenomata.<sup>41</sup> The adenoma shown in Figure 17 if it had a less well-defined capsule and did not exhibit so much compression of the surrounding tissue might be regarded as an unusually large island

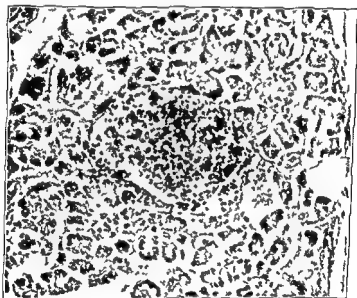


FIG 16 — Post mortem change simulating hemorrhage into island of Langerhans  
No 1638 Male aged sixty-eight years Nondiabetic

Thirty-eight cases in the earlier series showed a greater or less degree of hypertrophy of the islands of Langerhans. Usually not all the islands are hypertrophied but only a moderate proportion of them. However occasionally the majority may appear much enlarged. Islands were considered hypertrophic when they measured more than 400 micra in diameter. There is no correlation between the occurrence of hypertrophy, the age of the patient, the duration of the diabetes or the severity of the diabetes. In one instance markedly hypertrophic pancreas from a patient who died of carcinoma of the pancreas before death. In all other cases the diabetes was controlled. In the present case the diabetes was present in the tail of a pancreas which appeared secondary to a carcinoma which developed ten years before death. Then the

case became more or less insulin resistant and symptoms referable to the tumor became increasingly prominent.

Herxheimer<sup>16</sup> by ligating the pancreatic duct of chickens produced a marked increase in island tissue and a fivefold increase of insulin content. As this occurred the acinar tissue atrophied.

Huttl<sup>17</sup> noted large islands at autopsy in 3 diabetic patients on whom he had performed pancreatic ligation by the Mansfeld technique.

**Hemorrhage**—Hemorrhage into the islands has been described, but is probably either a post mortem change or an artifact, as a glance at Figure

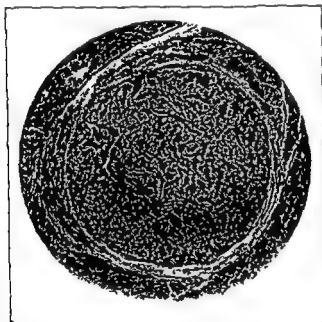


FIG. 1\*—Small adenoma of islet of Langerhans. Not capsule at 100 microns on surrounding tissue. 112b-177. Female, aged fifty-three years. Nondiabetic. Mallory's aniline blue stain.  $\times 40$ .

16 suggests. In only 2 cases was this hemorrhagic change at all noticeable and in one of these a man, aged seventy-two years, with diabetes of twenty-two years duration, cardiac decompensation was the cause of death with extreme passive congestion.

**Adenoma**—Adenoma of the islet of Langerhans was encountered only three times in this series.

Among the series of adenomas of the islands of Langerhans reviewed by one of us<sup>18</sup> there was only one, that of Heiberg<sup>19</sup> which occurred in a diabetic. Here there was a large mass of insular tissue 6 by 5 mm. in diameter,



which was found in the pancreas of a white female aged sixty-four years dying in diabetic coma.

In one of the cases reported by Smith and Seibel<sup>8</sup> the patient showed diabetic symptoms during an attack of lobar pneumonia prior to death.

Buchner<sup>21</sup> reported an insular adenoma in a male aged fifty-seven years whose diabetes present for two years terminated in hypoglycemic coma and death following injection of a small amount of insulin.

The association of insular adenoma and diabetes is apparently a rare one and the earlier cases were reported before the description of the newer differential stains for the islands. That the use of these stains is important

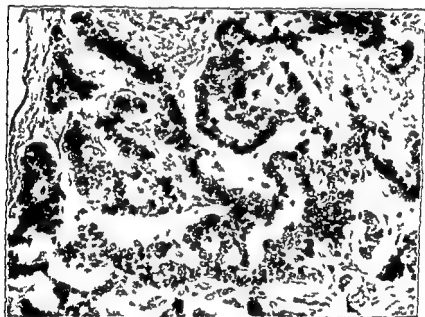


FIG. 18. Adenoma of Island of Langerhans. A09-101. Female aged forty-eight years. Nondiabetic. Mallory's anilin chlor. stain.  $\times 200$ .

is suggested by the case described by Hess<sup>5</sup> of a non-insulin producing carcinoma of islets. When studied with the modified Gros-Schultze method of Ferner the great majority of cells making up the tumor were silver positive and therefore presumably  $\alpha$  cells. The importance of such a finding in relation to the recent work on isolation of a hyperglycemic factor from the  $\alpha$  cells is obvious. Certainly in the future it will be desirable to study all such cases with the newer staining methods.<sup>22</sup> In addition to the Gros-Schultze method the latest techniques of Gomori and Tarruggen may be recommended (see Appendix A).

**Islands Apparently Normal**—Of particular importance from the theoretical as well as practical standpoint is the fact that a considerable number

of diabetic persons show no demonstrable pathology in the islands of Langerhans when studied by the usual methods. Out of 811 pancreases available for detailed study in this series 271 or 33 per cent, showed islands which were normal so far as could be determined by ordinary histologic means. A considerable proportion of these normal islands occurred in insulin treated cases that possibly might have shown hydropic change if they had not been so treated. However, a very fair number occurred in preinsulin days, or in cases which had received no or inadequate insulin. Also the great majority occurred before the newer differential stains of Lerner, Gomori, and others were available. No diabetic pancreas can now be said to have normal islands unless studied intensively with these new techniques, preferably also with some attempt to estimate the total mass of insular tissue (*see below*). However, there seems to be no question that such cases do exist.

The existence of these normal islands in diabetes mellitus has long been a puzzle to pathologists. It is rather difficult to conceive how we can have in insular diabetes with normal islands of Langerhans present, particularly in a disease that persists as long as life itself. This leads to one of four hypotheses: (1) the islands may be normal and insulin may be produced but it is either defective in quality or neutralized by some substance in the body; (2) the islands may be normal but their secretory activity inhibited; (3) the diabetes may be of other than insular origin; (4) the insulin may be formed normally by normal islands but through failure in transport the insulin is not brought into the blood stream and so not distributed to where its effects are needed. Whichever (if any) one of these hypotheses is true (No. 3 seems to be currently favored) we must face the fact that cases of diabetes absolutely identical with one another from the clinical standpoint show no evidence of structural disease in the islands of Langerhans in one instance and show marked injury of various types in others.

It remains for further investigation to establish how large is the group with normal islands when studied by *all available methods*, both qualitative and quantitative (*see page 62*). Until that time suggestions regarding the etiological significance of such factors as other endocrine glands or the liver must remain partly speculative.

Charrin<sup>24</sup> emphasizes the multiplicity and variability of the lesions that may be encountered in the pancreas in diabetes and points out that careful study must be undertaken before a pancreas is classified as "normal."

**Fatty Infiltration.** One minor change concerning the importance of which there has been a good deal of discussion is the presence of fat in the island cells. This was first pointed out by Dogiel.<sup>25</sup> In many cases of diabetes this can be demonstrated by either osmic acid or Sudan III or IV in many of the island cells, usually in considerably greater degree in the islands than in the acinar tissue. In sections stained with Sudan IV the islands may appear under low magnification as definitely orange spots. At one time this was considered to be a specific change characteristic of dia-

betes Weichselbaum and Stangl<sup>56</sup> found in their material that diabetics showed more fat in the islets of Langerhans at autopsy than did those dying at the same ages from other disease conditions. The work of Summers<sup>57</sup> showed that the presence of fat in the islets was not characteristic of diabetes but was present in other diseases notably alcoholic intoxication in which it was particularly prominent. In fact Summers felt that

islets in diabetic than in nondiabetic persons was probably a result rather than a cause of diabetes. In its most striking form the islets



FIG. 19.—Pancreas showing lipomatosis and moderate fatty infiltration of islet epithelium. D426-35. Female aged fifty-four years. Duration of diabetes five years. Sudan IV stain.  $\times 310$ .

appear in Sudan stained sections as deep orange-red spots. Wilder found marked fatty infiltration of 11 cases moderate in 15. Table 6 shows the degree of fatty infiltration in a number of pancreases from our series both diabetic and nondiabetic.

TABLE 6 — FAT IN THE ISLANDS OF LANGERHANS

	Degree of infiltration		
	Slight	Moderate	Severe
Diabetic cases	0	18	13
Non diabetic cases	1	2	6

Apparently there is no significance to be attached to this lesion. An example of moderate infiltration is shown in Figure 19.

It is interesting to compare fatty change with glycogen infiltration of the islands (see page 46). Apparently either substance may or may not be associated with vacuolation of the cytoplasm.

TABLE 7 — QUALITATIVE ISLAND CHANGES IN DIABETES MELLITUS

Author	Tetrasaccharide	Glycogen	Mucin	Fatty acids	Hydrophobic change	Protein change	Hemorrhage	Lymphocytic infiltration	Hypertrophy
Cecil	90	0	0	49	0	0	0	1	41
Willis <sup>44</sup>	29	8	4	12	0	0	0	5	0
Gibb and Logan <sup>45</sup>	142	11	30	29	0	15	7	0	0
Hersheimer <sup>46</sup>	9 <sup>a</sup>	2 <sup>a</sup>	3 <sup>a</sup>	17	0	0	0	0	0
Warren and LeCompte	811	271	333	186	32	20	16	11	63
Total	1109	312	437	339	32	35	25	17	104

\* Only those of Willis's cases are included in which the enlargement was specifically described.

## QUANTITATIVE VARIATIONS IN THE ISLANDS OF LANGERHANS

Enumerating the islands in the human pancreas is extremely difficult and for practical purposes only rough estimates are possible based on the total number of islands in a number of representative sections taken from different portions of the gland. Any method such as this of course is exposed to numerous errors and presumably the statements as to the number of islands occurring in a given human pancreas are all open to question. The methods worked out by Bensley<sup>47</sup> and his students for enumeration of the islands in experimental animals are extremely valuable there but are rarely applicable to human tissues. Clark<sup>48</sup> working in Bensley's laboratory was able to count fairly accurately the number of islands in the pancreas of seven human adults. He found the number to range from 120,000 to 1,700,000. As noted in Chapter 2 Ogilvie found values in infants ranging from 117,226 to 2,325,123 and Bargmann<sup>49</sup> records counts in 5 adults varying from 208,369 to 1,760,000. (See Chapter 2 for Ogilvie's data on the weight of the normal island tissue.)

betes Weichselbaum and Stangl<sup>16</sup> found in their material that diabetes showed more fat in the islands of Langerhans at autopsy than did the individuals at the same ages from other disease conditions. The work of Summers<sup>17</sup> showed that the presence of fat in the islands was not characteristic of diabetes but was present in other diseases notably alcoholic intoxication in which it was particularly prominent. In fact Summers felt the lesion was to some extent characteristic of prolonged alcoholic intoxication.

Wilder<sup>18</sup> felt that this lesion if it can be called such although more frequent in diabetic than in nondiabetic persons was probably a result rather than a cause of diabetes. In its most striking form the islands

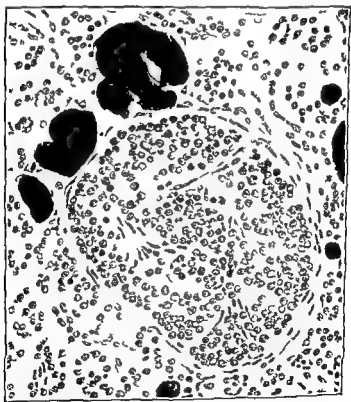


FIG. 19.—Pancreas showing lipomatosis and moderate fatty infiltration of islet epithelium. DA26-35. Female aged fifty-four years. Duration of diabetes five years. Sudan III stain.  $\times 310$ .

appear in Sudan stained sections as deep orange-red spots. Wilder found marked fatty infiltration of 11 cases, moderate in 15. Table 6 shows the degree of fatty infiltration in a number of pancreases from our series, both diabetic and nondiabetic.

TABLE 6—FAT IN THE ISLANDS OF LANGERHANS

	Degree of infiltration		
	Mild	Moderate	Severe
Diabetic cases	0	18	13
Nondiabetic cases	1	2	6

Apparently there is no significance to be attached to this lesion. An example of moderate infiltration is shown in Figure 19.

It is interesting to compare fatty change with glycogen infiltration of the islands (see page 46). Apparently either substance may or may not be associated with vacuolization of the cytoplasm.

TABLE 7—QUALITATIVE ISLAND CHANGES IN DIABETES MELLITUS

Author	Total cases	Vacuol	Glycogen	Fibrosis	Hypertrophy	Islet change	Islet atrophy	Hemorrhage	Islet necrosis	Lymphocytic infiltration	Hypertrophy
Cecil <sup>1</sup>	90	0	17	43	0	0	2	1	41		
Wilber <sup>2</sup>	29	8	4	13	0	0	0	5	0		
Cobb and Logan <sup>3</sup>	14	11	30	79	0	15	7	0	0		
Hersilemer <sup>4</sup>	9	0	0	11	0	0	0	0	0		
Warren and LeCompte	811	271	717	147	7	20	16	11	63		
Total	1169	312	472	379	32	15	25	17	104		

\* Only those of Wilber's cases are included in which the pancreas was specifically described.

## QUANTITATIVE VARIATIONS IN THE ISLANDS OF LANGERHANS

Enumerating the islands in the human pancreas is extremely difficult and for practical purposes only rough estimates are possible based on the total number of islands in a number of representative sections taken from different portions of the gland. Any method such as this of course is

the islands in experimental animals are extremely valuable there but are rarely applicable to human tissues. Clark<sup>5</sup> working in Bensley's laboratory was able to count fairly accurately the number of islands in the pancreas of seven human adults. He found the number to range from 120,000 to 1,760,000. As noted in Chapter 2 Ogilvie found values in infants ranging from 117,226 to 2,325,123 and Bargmann's<sup>6</sup> records counts in 5 adults varying from 208,399 to 1,760,000. (See Chapter 2 for Ogilvie's data on the weight of the normal island tissue.)

to imperfect technique  
found diminished numbers in 13 cases more.  
Among Cecil's 90 cases 20 showed few islands as did all of Weil  
and Stangl's 18 cases

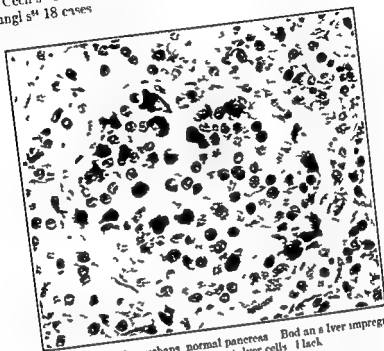


FIG. 20.—Island of Langerhans normal pancreas Bodan's liver impregnation  
after Bouin fixation Islet cells black

Kraus<sup>46</sup> reported that the number of islets per 50 cu mm of pancreas in 20 diabetics varied from 8.7 to 128 with an average of 54.1. This he stated gave a ratio of normal to diabetic of 2.4 to 1. Susman<sup>47</sup> using camera lucida drawings estimated the area of islet tissue in proportion to pancreatic tissue in each of 10 microscopic fields in 202 cases including 51 diabetics. Of 51 diabetics 29 had islet area readings below 0.9 per cent 14 were from 0.9 to 1.3 per cent 2 from 1.4 to 1.7 per cent 4 from 1.8 to 2.0 per cent 1 from 2.7 to 3.6 per cent and 1 was over 4.0 per cent. He considers the range 0.9 to 2.7 per cent as normal for adults and 0.9 to 3.6 per cent as normal for infants. Values over 3.6 per cent he thought were likely to be associated with hypoglycemia. The results are of interest but one may question whether counting the islands in only 10 microscopic fields is sufficient to give an accurate picture of the mass of insular tissue in a given pancreas. Ogilvie<sup>48</sup> warns that reduction of islet tissue is still unproven as a factor of genuine etiological significance especially in view of the fact that experimentally one-eighth of the pancreas is enough to prevent diabetes.

Congenital hypoplasia of the pancreas very rarely occurs (6 cases, 3 associated with diabetes mellitus)<sup>67</sup> and is apparently due to absence of the dorsal pancreatic anlage.

Moore<sup>68</sup> has reported a striking case of aplasia of the islands occurring in a thirteen year-old girl whose diabetes had existed at least six years. Numerous sections of the pancreas revealed no normal islands, only a few small cell groups suggesting isular cells being present. Sections of the

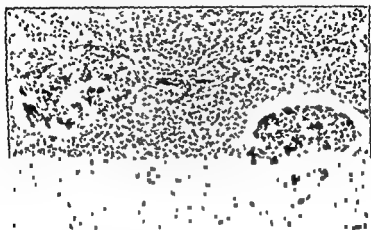


FIG. 21. Two islets of Langerhans in a diabetic pancreas. Frozen section. Grocott's fast green stain. Islet cells black.  $\times 100$ .

pituitary and hypothalamic regions were essentially negative. Dr Lukens' case is described in Chapter 18.

Binger and Keith<sup>69</sup> reported an extraordinary case of fibrosis of the pancreas with generalized edema. No islands were found (special stains apparently were not used) and yet the patient was not diabetic.

As regards modification of the total mass or volume of the islands by various stimuli, the experimental results of Huest et al.<sup>70</sup> are of great interest. They used Binkley's method of vital staining of the islands with neutral red, then estimated the volume by pressing the pancreas between glass plates, projecting the islands on a screen and tracing the area with a planimeter. From this they calculated the total weight of the isular tissue. They found that the weight of the islets increases with age in the rat. Factors reducing islet growth were (1) undernutrition, (2) insulin administration, and (3) hypophysectomy. Procedures stimulating islet growth were (1) continuous injection of glucose, (2) injection of crude extracts of pituitary gland, and (3) administration of thyroid extract.

The important critical work of Tejning<sup>71</sup> on methods for ascertaining the volume of the islet organ, and the results of feeding various diets are described in Chapter 2.



It would seem desirable to combine some of these methods for calculating the total volume or mass of islet tissue with the differential stains mentioned in the next section. Obviously it would be useful to know whether an increase or decrease of insular mass involved predominantly the  $\alpha$  cells or the  $\beta$  cells.

**Differential Counts of Cells of Islands**—It is only in recent years with the development of reasonably satisfactory staining techniques applicable to human post mortem material, that attempts have been made to do differential counts of the various cell types in diabetes. Foremost among the workers in this field is Gomori<sup>23</sup> who in 1941 described the chrome-alum hematoxylin and phloxine stain which is still perhaps the best available method for differentiation of  $\alpha$  and  $\beta$  cells. In the same paper this author

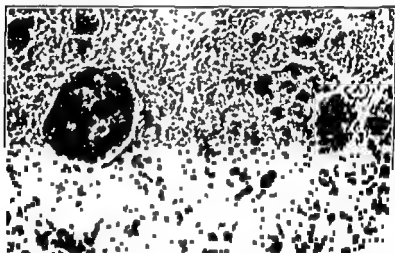


FIG. 22.—Pancreas of 50-year old woman. Diabetes unrecognized before death. Cross-hatched stain on frozen section showing two islets consisting almost entirely of  $\alpha$  cells (black).  $\times 150$

described differential counts done on several diabetic pancreases by his method. In some of these there appeared to be a definite reduction in the ratio of  $\beta$  to  $\alpha$  cells but the results were considered of questionable significance.<sup>23, 24</sup> More recently Gomori<sup>25</sup> writes that he has done differential counts on the islets in over 50 cases of diabetes and has obtained normal differential counts in 60 per cent. His most recent staining technique<sup>26</sup> (see Appendix) may prove of great value in this respect.

Bell<sup>27</sup> applied Gomori's chrome-alum hematoxylin method to 30 diabetic pancreases and found the  $\beta$  granules to be completely absent in 11 of these and present in very small quantity in 10 others. However 2 cases with normal granulation were instances of clinically severe diabetes. Although admitting that the significance of the  $\beta$  granules is still not clear

Bell<sup>18</sup> concluded that a histological diagnosis of diabetes could be made in this way in about 80 per cent of cases. Degranulation of the  $\beta$  cells is readily produced experimentally by hyperglycemia (Barron<sup>19</sup> Peterson<sup>20</sup>) and hence it seems possible that it may have no more specific significance than this when encountered in the human pancreas but may be useful in diagnosis in the same way that glycogen in the renal tubules is useful.

Hartroft<sup>21</sup> using phase contrast microscopy suggests that under certain conditions the size of the granules may provide a more sensitive index of the endocrine activity of the  $\beta$  cell than the number of granules.

Outstanding among the recent students of the subject is Lerner<sup>22-24</sup> who, as noted in Chapter 2, has applied the Gros-Schultze silver stain to the islands and on the basis of his findings has formulated a theory which holds that the silver cells (including the  $\alpha$  cells) are the precursors of the islands (inselpotents). According to this point of view the  $\alpha$  cells represent immature (unreifen) elements which are capable of differentiating into  $\beta$  cells under proper stimulation. According to Lerner this accounts for the apparent increase in islands with absence of symptoms in the newborn infant as compared with the adult (the infant's islands are predominantly  $\alpha$  cells).

Lerner has applied the Gros-Schultze stain to the diabetic pancreas with interesting results. In only 3 of 17 diabetics was he unable to demonstrate an increase in silver cells. (In addition to the  $\beta$  cells of the islands he includes cells scattered among the acini and in the ducts and states incidentally that the latter cells are not well demonstrated by other staining techniques). He seems to be convinced that the increase in  $\alpha$  cells is an absolute not a relative one and interprets this phenomenon as indicating an attempt at regeneration thus confirming his hypothesis that the  $\alpha$  cells are precursors of the  $\beta$  cells. As additional evidence he finds buds of silver cells especially in diabetic children projecting from the ducts (Inselprossen and Inselzapfen). He describes in detail the case of a four year old diabetic child whose pancreas showed numerous islands and island buds almost completely composed of silver cells.

These observations led Lerner to formulate the hypothesis that diabetes, especially the juvenile type, is fundamentally due to a failure of silver cell (including the  $\alpha$  cells) to ripen into  $\beta$  cells, i.e. he conceives of diabetes as essentially due to a *maturational arrest* of the cells of the islands. From analogy with the increase of silver cells which he was able to produce in the experimental animal, he suggests that the disease may be due to

means of stimulating the silver cells to mature into insulin producing elements.

Lerner's concept whether or not it is borne out by subsequent work is provocative and worthy of careful consideration. One wonders if his maturational arrest if indeed it exists might not be analogous to similar situa-

tions affecting the bone marrow due to a deficiency (e.g. pernicious anemia) or to 'toxic' factors (benzol, other drugs). Certainly the inability to form new  $\beta$  cells would seem to be the fundamental defect in the diabetic patient.

As noted in Chapters 2 and 2b, Lerner has recently modified his earlier view to some extent to take cognizance of the newer thoughts regarding possible production of the H.G. factor or glucagon by the  $\alpha$  cells and has indulged in interesting speculation on the possible role of the  $\alpha$  cells not only in normal carbohydrate metabolism but in cases of insulin resistance.

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TABLE 8 DIFFERENTIAL COUNTS ON THREE PANCREASES

	Total cells in 20 islets		Ratio $\beta/\alpha$
	$\beta$	$\alpha$	
A50-40 (normal)	1362	167	8.4
A50-38 (diabetic)	791	214	4 "
U50-5 (diabetic)	561	210	2 "

Variation in the size of the islands in the three cases is of course indicated by the considerable differences in the total number of cells in 20 islands in each instance. Obviously a great deal more work should be done along these lines.

What appears to be a careful piece of work. Terbruggen<sup>88, 89</sup> tried to

Appendix A) The number of  $\beta$  cells in the islets of Langerhans in the pancreas of nondiabetics while in 80 per cent of diabetics the ratio was less than 5

Table 9 is reproduced from Terbruggen<sup>45</sup> representing his counts on 64 nondiabetics and 26 diabetics of varying ages (but all over sixteen)

TABLE 9.—RANGE OF DIFFERENTIAL COUNTS IN 64 NONDIABETICS AND 26 DIABETICS (from Terbruggen<sup>45</sup>)

	Diminution of $\beta$ cells		Normal range		Increase of $\beta$ cells	
Ratio of $\alpha$ to $\beta$ cells	1:1 to 1:2	1:2 to 1:3	1:3 to 1:4	1:4 to 1:5	1:5 to 1:6	1:6 to 1:7
Non-diabetics	0%	23%	21%	21%	15%	4%
	21%		58%		11%	
Diabetics	15%	42%	12%	8%	0%	0%
	50%		20%		0%	

It is obvious that there is some overlapping. However he holds that the ratio is never above 5 in a diabetic and in this respect agrees with Hess.<sup>42</sup> The question arises as to whether a reduced ratio of  $\beta$  to  $\alpha$  cells represents (a) an absolute decrease in  $\beta$  cells or (b) a *relative* decrease in  $\beta$  cells i.e. an increase in  $\alpha$  cells. The latter possibility is indicated by Lerner must be considered particularly in juvenile diabetics. Terbruggen thought the islets were usually reduced in number in the diabetic and in many cases the weight of the pancreas as a whole seemed to be reduced. He therefore concluded that probably the whole island apparatus of the diabetic contains fewer  $\beta$  cells than that of nondiabetics. Terbruggen does not accept without reservation the hypothesis of Lerner that the  $\alpha$  cells are precursors of the  $\beta$  cells: he points to the frequency of mitotic figures in  $\beta$  cells and also to studies of his own showing that in insular adenomas  $\beta$  cells may arise from nongranular precursors thus indicating that  $\alpha$  cells are not obligatory precursors of  $\beta$  cells. He suggests that the  $\alpha$  cell may have a special function of its own.

We have tried the acid fuchsin and methyl green method as described by Terbruggen with some success. However all the aniline dye techniques seem subject to slight variations in color values which at times make differential counts difficult or impossible. Herein lies the great advantage of the Gross-Schultze stain when properly carried out: the clarity of the picture facilitates counting although it must be admitted that this advantage is partly counterbalanced by the thickness of the frozen sections also by the capriciousness of the stain.

Cromi's<sup>46</sup> new aldehyde-fuchsin stain for the  $\beta$  cells shows great promise and may prove to be the most generally satisfactory method for differential counts yet devised.

## THE PANCREAS IN DIABETES MELLITUS

tions affecting the bone marrow, due to deficiency (e.g. pernicious anemia) or to toxic factors (benzol other drugs). Certainly the inability to form new  $\beta$  cells would seem to be the fundamental defect in the diabetic patient.

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TABLE 8 — DIFFERENTIAL COUNTS ON THREE PANCREASES

	Total cells in 20 islets		Ratio $\beta/\alpha$
	$\beta$	$\alpha$	
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A50-38 (diabetic)	998	214	4
A50-3 (diabetic)	561	210	2.7

Variation in the size of the islands in the three cases is of course indicated by the considerable differences in the total number of cells in 20 islands in each instance. Obviously a great deal more work should be done along these lines.

In what appears to be a careful piece of work Terbruggen<sup>28,29</sup> tried to confirm Lerner's results using not only the Gros-Schultze method but also a simple modification of Bensley's acid fuchsin methyl green method (see Appendix A). He found that the  $\beta/\alpha$  ratio was between 3 and 5 for most nondiabetics while in 80 per cent of diabetics the ratio was less than 3.

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	1:1 to 1:2	1:2 to 1:3	1:3 to 1:4	1:4 to 1:5	1:5 to 1:6	1:6 to 1:"
Nondiabetics	0%	21%	21%	21%	13%	4%
	21%		58%		19%	
Diabetics	38%	42%	12%	8%	0%	0%
	80%		20%		0%	

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Ratio of $\alpha$ to $\beta$ cells	Distribution of $\beta$ cells		Normal range		Frequency of $\beta$ cells	
	1:1	1:2	1:3	1:4	1:5	1:6
	to	to	to	to	to	to
	1:2	1:3	1:4	1:5	1:6	1:7
Nondiabetics	0%	23%	29%	21%	15%	4%
	23%		50%		19%	
Diabetics	38%	42%	12%	8%	0%	0%
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Creutzfeldt<sup>30</sup> in a study of alloxan diabetes in the dog including many observations on the histo-physiology of diabetes in general remarks on the variation in the results of differential counts by different authors and notes the difficulty just mentioned of counting cells in thick frozen sections (Hess also mentioned an error of at least 10 per cent in his counts). Creutzfeldt found no evidence in the dog to support Kerner's thesis that  $\alpha$  cells can be transformed into  $\beta$  cells. He observed no evidence of such transformation in the alloxan treated dog and thought that since the  $\alpha$  cells persist apparently undamaged they must have a function. He obtained a  $\beta/\alpha$  cell ratio of 8:1 for the normal dog.

To summarize the newer work on differential counts the varying figures for the normal  $\beta/\alpha$  cell ratio obtained by various authors may be assembled: Kerner 5:1, Hess 9:1, Terbruggen 4:1, Gomori 3:2 to 9:1. Gomori's recent statement<sup>31</sup> that 60 per cent of diabetics may have normal differential counts should also be mentioned again. The differences are unexplained; they may be due to staining techniques, quantity or quality of material, methods of counting (especially on frozen sections) or other unknown factors. Certainly since most authors found some normals in the diabetic range and often some diabetics in the normal range it seems obvious that a post mortem diagnosis of diabetes cannot be made by this method with 100 per cent accuracy as Hess seems to suggest. How often such a diagnosis can be made remains for further investigation to tell; no doubt it is worth trying some of the methods mentioned in questionable cases since a very low  $\beta/\alpha$  ratio is certainly almost conclusive evidence of diabetes (see Chapter 27).

Surprisingly little work has been done (probably in part because of the tedious methods required) on the total mass of island tissue both absolute and in relation to the weight of the pancreas. It would seem desirable to apply techniques such as those of Ogilvie<sup>43</sup>, Haist<sup>44</sup> and Su-min<sup>45</sup> to the human diabetic pancreas in conjunction with the differential stains mentioned above in order to establish the presence or absence of a reduction in the total mass of  $\beta$  cells (and perhaps increase in  $\alpha$  cells) in human diabetes. Conceivably the total volume of island tissue might be reduced to such an extent in some cases that a normal differential count might be associated

(  $\beta/\alpha$  cell )

more or less opposes that of insulin it will be essential to estimate the quantities of both types of cells.

It would be desirable also to correlate quantitative studies on the two types of cells in the islets with the recent work of Wrenshall *et al.*<sup>46</sup> on the extractable insulin of the pancreas. These investigators found very

little insulin in the pancreases of severe diabetics with onset in youth ('growth-onset' type) and relatively larger amounts in subjects who had reached full stature prior to discovery of diabetes ('maturity-onset' type). This important work is considered further in Chapter 20.

### CHANGES INVOLVING THE PANCREAS AS A WHOLE

Aside from the insular changes, changes in the pancreas as a whole must be considered, particularly those involving the acinar cells.

**Fibrosis**—Fibrosis is perhaps the simplest and most frequently occurring change. Normally there is only a relatively delicate fibrous tissue septum

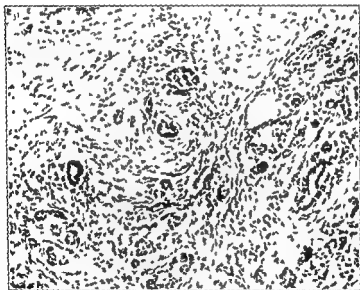


FIG. 23—Marked fibrosis of pancreas, both interlobular and interacinar. (X 150).  
Male, aged seventy years. Duration of diabetes thirty-nine years.

between the lobules of the pancreas and there is a delicate basement membrane rarely more than about 1 micron thick between the acini and the islands. Fibrosis, both interlobular and interacinar, is not infrequent. It may or may not be accompanied by a moderate degree of round-cell infiltration, either lymphocytes or large mononuclear cells. This change may vary from irregular patches of fibrous tissue scattered through the pancreatic substance, often tending to center about the ducts, to the almost complete disappearance of the acinar tissue with dilatation of the ducts characteristic of obstruction, such as one sees in ligation experiments.

Of the 405 cases that showed varying degrees of fibrosis 189 showed slight fibrosis the bulk of which was interacinar 141 showed moderate fibrosis, again the bulk of which was interacinar rather than interlobular, and 74 showed marked fibrosis, again predominantly of the interacinar type.

There is no definite relationship between the occurrence of island lesions and interacinar fibrosis. However, severe cases of either hyalineization or fibrosis of the islands usually show at least a moderate degree of interacinar fibrosis.



FIG. 24.—Extreme lipomatosis of pancreas. Note white epithelial tissue in bed of large mass of fat. Fat has been stained with Sudan IV to provide better contrast. No. 1870. Female, aged sixty-five years. Duration of diabetes eight months. Natural size.

Vartiainen<sup>11</sup> found gross fibrosis of the pancreas 30 times in a series of 166 diabetics and not at all in a control series of the same number.

**Lipomatosis.**—Fatty infiltration or lipomatosis of the pancreas as a whole has been considered as characteristic of diabetes. In Figure 24 is shown the extreme degree of fatty infiltration which can occur in a pancreas. Here hardly any epithelial tissue remains, the organ being converted into little more than a mass of fat and fibrous tissue. The amount of pancreatic fat is very closely related to that of the normal body fat and probably represents simply the degree of fat storage rather than being associated with the diabetic process in any peculiar way. Certainly marked lipomatosis is common in obese patients in the absence of diabetes. (See Chapter 4.)

Two hundred forty-one, or 30 per cent, of the cases in our series showed varying degrees of lipomatosis, ranging from a slight deposit of fat among the acini and along the interlobular septa to an extremely marked riddling

of the pancreas with large and small deposits of fat frequently occurring in single cells.

Incidentally it hardly need be remarked that the weight of a pancreas such as that illustrated in Figure 24 is of no significance unless the fat is extracted.

**Arteriosclerosis.** Marked sclerosis of the pancreatic vessels occurred in 10 cases. Arteriosclerosis of the pancreas is not nearly so common as is arteriosclerosis of the spleen which lies in the same vascular circuit. While the splenic artery may be extremely sclerosed, calcified and tortuous, there frequently is relatively little change in its branches which lead



FIG. 24.—(Symington, D. A. 2° 9.) Female aged sixty years. Duration of diabetes five years. Note large amount of periductal fat and small cell fat cells throughout acinar tissue. Note also large number of hyaline islets. (Hosp. Path. Dept., McGill Univ. Med. Sch.) X 225.

to the pancreas and there frequently is very little change in the pancreaticoduodenal artery. With only 7.4 per cent of the pancreases showing a marked degree of arteriosclerosis, arteriosclerosis is a factor in the production of diabetes while it cannot be ruled out as hardly of outstanding importance. Moschowitz<sup>27</sup> seems to be practically alone in maintaining that arteriosclerosis (if the hyperplastic type) may be of importance in leading to hyalinization of islets and causing diabetes in persons in the older age



FIG 26 —Marked fibrosis and lipomatous of pancreas with practical disappearance of acinar tissue. Note marked dilatation of ducts and large number of islands present in the fibrous tissue. Change secondary to carcinoma of the head of the pancreas. U21-33. Female, aged sixty nine years. Duration of diabetes eleven years. Fown methylene blue stain.  $\times 15$

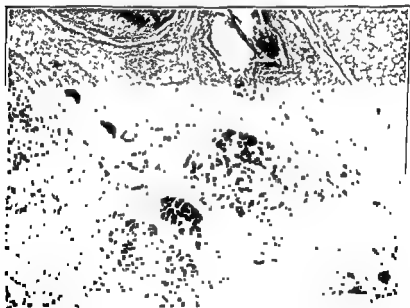


FIG 27 —Pancreas showing lower corner (66)

rows and  
lactie I

te mucous glands in k ft  
se stain  $\times 15$

**Pancreatitis**—Acute pancreatitis appeared as a causal factor in 5 cases of this series. Here we have the development of diabetes simply through destruction of a considerable proportion of the pancreatic tissue just as one might surgically remove a sufficient amount of the pancreas to cause



FIG. 28.—Longitudinal section of pancreas from case of acute hemorrhagic pancreatitis. Note white fat necrosis. No. 537. Male, aged seventy-one, years. Nondiabetic.

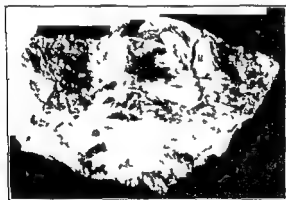


FIG. 29.—Section through head of pancreas from same case as Figure 28 showing marked necrosis and hemorrhage. Natural size.

the development of diabetes experimentally. The excessively rare authentic cases of post-traumatic diabetes such as Wells' case<sup>22</sup> probably rest on destruction of insular tissue in the course of pancreatitis due to the trauma.

Probably cases of diabetes after acute pancreatitis would be more frequent were it not that the lesions which destroy enough of the pancreas to

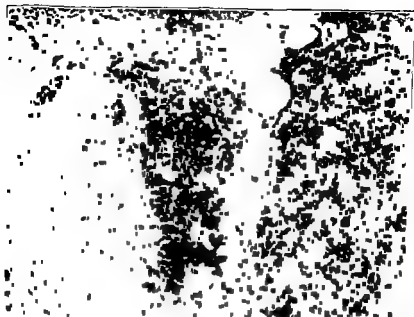


FIG. 30 —Acute pancreatitis showing necrosis of pancreatic tissue, fat necrosis, fibrin deposits and leukocytic infiltration. No. 537. Male, aged seventy-one years. Non-diabetic. Iron hematoxylin Van Gieson stain.  $\times 150$ .



FIG. 31 —Acute pancreatitis, periductal. Note polymorphonuclear leukocytes within ducts and infiltration of wall. A14-79. Tissue methylene blue stain.  $\times 110$ . (68)

produce insulin deficiency are generally sufficiently severe to cause death from the pancreatitis itself, before there has been opportunity for diabetes to demonstrate itself.

A most interesting series was presented by Bernhard<sup>21</sup> who studied 50 cases of operative recovery from acute pancreatitis. Five developed diabetes, 3 dying of the disease and 2 living with severe diabetes. The time interval between pancreatitis and diabetes ranged from immediately subsequent to thirteen years.

Gumbill *et al*<sup>22</sup> report a case of diabetes associated with acute hemorrhagic pancreatitis.

Increasing awareness of the possible relation between chronic pancreatitis and diabetes is evident in the recent literature. Comfort *et al*<sup>23</sup> found diabetes in 7 of their 29 cases of chronic relapsing pancreatitis. Maimon *et al*<sup>27</sup> obtained a diabetic type of glucose tolerance curve in 11 of 9 cases of chronic pancreatitis suggesting that this test might uncover many more cases if used more often. Little attention seems to have been paid to the possibility that bumps may account for a large percentage of cases of chronic pancreatitis.<sup>24, 25, 100</sup> Shumacker<sup>101</sup> and Paxton and Payne<sup>102</sup> call attention to alterations in glucose tolerance in acute pancreatitis.

Also as emphasized by Root<sup>103</sup> it is probable that more consideration should be given to acute pancreatitis as a precipitating cause of coma in a recognized diabetic.

This series includes in addition to the 5 cases where pancreatitis was apparently the cause of their diabetes 12 cases of pancreatitis developing six months to thirteen years after the onset of diabetes. Two showed pancreatic apoplexy, four showed acute pancreatitis and six subacute pancreatitis.

It must be true of course that some of the cases of fibrosis mentioned above represent healed pancreatitis but there would seem to be no means of determining the incidence other than by exhaustive clinical investigation.

**Abscesses**—Four cases of metastatic abscesses in the pancreas were encountered all in persons suffering from staphylococcus pyemia. No case has been encountered since the first edition.

**Syphilis**—Pancreatic changes secondary to syphilis may cause diabetes but only rarely. Among 500 diabetic autopsies reported by Simmonds,<sup>104</sup> only 3 cases were found where the small fibrotic pancreas damaged by syphilis had led to diabetes. In neither of his 2 cases of gumma of the pancreas was glycosuria noted. A case of syphilis studied by Carnot and Harvier<sup>105</sup> died with severe diabetes and showed at autopsy syphilitic pancreatitis and gumma. Lamont<sup>106</sup> found but few cases of coexisting syphilis and diabetes.

**Calculi**—In our early series of 127 autopsied diabetics pancreatic calculi were encountered three times. Walder<sup>28</sup> reported 3 cases among 55 autopsied cases. One of his cases showed hemochromatosis also, another gall



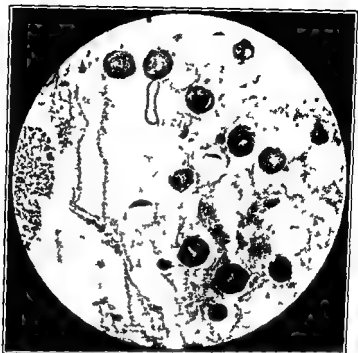


FIG 32 —Pancreatic cysts with concretions A17-67 Nondal et al. X 225

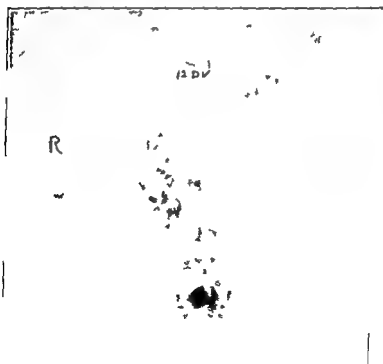


FIG 33 —Roentgenogram showing pancreatic calcifications  
Duration of disease years

and twenty three years  
17

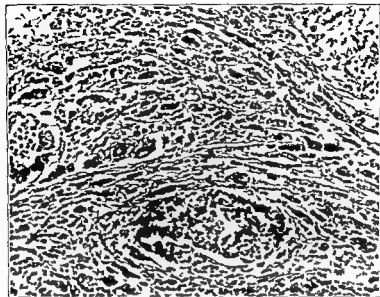


FIG. 31.—Carcinoma of pancreas showing persistence of islands in the midst of tumor tissue. No. 1638. Male aged sixty-eight years. Nondiabetic. Phosphotungstic acid hematoxylin stain.  $\times 190$ .

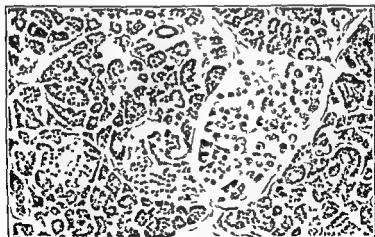


FIG. 32.—Carcinoma of islet-cell type showing appearance of islands of Langerhans. Eosin-methylene blue stain.  $\times 250$  (Drawing).

stones and the third cholecystitis. Morrison and Bogan<sup>107</sup> have pointed out that the diagnosis can be made during life by roentgenogram. Figure 36 taken from one of their plates shows multiple calculi in a diabetic girl aged twenty three years. They reported 8 cases of which 4 were diabetic.

Fischer<sup>108</sup> in a review of the literature stated that about half of 270 reported cases of pancreatic lithiasis had diabetes. Edmondson *et al*<sup>109</sup> reported 30 per cent of 26 cases as having diabetes.

The presence of calculi naturally predisposes to fibrosis of the pancreatic tissue, either through direct obstruction or tendency to localized infections with subsequent scarring. In obstruction the effect is closely akin to that of duct ligation resulting in atrophy of the acinar tissue and persistence at least for a considerable time of the islands. This lesion is of much historical interest as one case reported by Barron<sup>110</sup> aroused Banting's interest and thus played a part in the development of insulin.

Diffuse calcification of the pancreas without calculi is apparently an exceedingly rare condition. Nuzum<sup>111</sup> found 17 cases in the literature with evidence of disturbed carbohydrate metabolism in 45 per cent. Present and Greyman<sup>112</sup> review the radiological findings.

**Amyloidosis**—Since reactions characteristic of amyloid were not infrequently obtained in cases of hyalinization of the islands much attention was paid to the occurrence of amyloidosis and involvement of the pancreas as part of a more or less general amyloid deposition in the usual organs (so-called typical amyloidosis). This was found in only 2 cases. One a female aged sixty two years died of carcinoma of the uterus with diabetes of two years duration. Calculi were also present in the pancreas. The second a female aged fifty four years whose diabetes had been present for 49 years died of multiple abscesses and staphylococcus pyemia.

For a discussion of the relation of atypical amyloid to hyalinization of the islets see page 33.

**Carcinoma**—While carcinoma is of relatively infrequent occurrence among diabetics (672 fatal cases among 8384 diabetic deaths reported by Joslin<sup>113</sup>) carcinoma of the pancreas is not a rare lesion compared with other types of carcinoma. Whereas the average incidence of carcinoma of the pancreas is only 4 per cent of all malignant tumors it was found by McKittrick and Root<sup>114</sup> in 32.4 per cent of the 37 cases of malignant disease in diabetics seen at the New England Deaconess Hospital during three and half years.

Urnv *et al*<sup>115</sup> have reported a case of mild diabetes in a male aged forty six years developing two and a half years subsequent to the appearance of a slowly growing carcinoma of the pancreas. In the 2 cases of Burkhardt<sup>116</sup> with carcinoma of the head of the pancreas obstructing the ducts clinical diabetes occurred in spite of the presence of regenerating island. Benson and Gordon<sup>117</sup> found diabetes in 6 of 28 patients with cystadenoma of the pancreas.

For a consideration of cancer in diabetes and especially carcinoma of the pancreas as an etiological factor see Chapter 24

**Metaplasia of Duct Epithelium**—This change ranging from a mere heaping up of clusters of basal epithelial cells to complete keratinization is not related to diabetes. Seen at its best in certain cases of A avitaminosis and "fibrocystic disease" it may be associated with retained secretion calculi or other duct abnormalities. In this series 19 instances were encountered among 811 pancreases examined microscopically without significant age or sex distribution the youngest being a woman of fifty nine. Korpas<sup>11</sup> found this change in 58 of 500 human pancreases.

**Quantitative Variations**—So far as the size of the pancreas itself is concerned there is relatively little indication one way or another as to the role that this plays. Herthelmer<sup>12</sup> noted the pancreas to be small thin or heavily infiltrated with fat in 105 of 162 cases of diabetes mellitus. Of the 730 cases of this series in which the weight of the pancreas was known there were 122 cases in which the pancreas was noted as definitely small or less than 50 grams, 481 cases in which the pancreas was normal or between 50 and 100 grams and 127 cases in which the pancreas was large over 100 grams. Of course the weight must be accepted with more than a grain of salt as the degree of fatty infiltration can only be estimated and a pancreas for example may weigh as much as 240 grams and yet contain less pancreatic tissue than one weighing perhaps 50 grams.

In a group of 31 pancreases from diabetic patients Terplan<sup>13</sup> found 8 to be fatty. The weights ranged from 21 grams in a child fifteen years of age and 22 grams in an adult of fifty-two years to 110 grams.

Vartiainen<sup>14</sup> in a careful gross study of the organs of 166 diabetics matched with controls of comparable age and sex found that 22 of the diabetics had pancreases weighing less than 40 gm. while only 2 controls had pancreases of less than 40 gm.

Terbruggen<sup>15</sup> encountered small pancreases (17 to 40 gm.) quite frequently in his juvenile diabetic group.

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## Chapter 4

# LESIONS OF THE PANCREAS IN NONDIABETIC INDIVIDUALS

To serve as a control for the lesions of the pancreas in cases of diabetes discussed in the last chapter the pancreas was studied in 200 consecutive autopsies performed on nondiabetic individuals in this laboratory in 1928 and a portion of 1929. The findings in the first 150 of these have been reported.<sup>1</sup> The primary cause of death ranged from such chronic conditions as carcinoma, multiple sclerosis, and endocarditis to pneumonia, meningitis, and postoperative pulmonary embolus. These cases are fairly representative of various ages ranging from one day to eighty-seven years at the time of death. However, it may be seen from the tables they are rather weighted in the later decades. Thus the age distribution is not unlike that of diabetic deaths. In only 6 cases were pancreatic changes an important factor in the clinical course or the causation of death. So much attention has been paid to even minor lesions in the pancreas of diabetics that a tendency has developed to relate complacently almost all types of pancreatic lesions with diabetes.

**Methods.**—Sections of the pancreas were fixed routinely in Zenker's fluid and stained with phloxine-methylene blue and phosphotungstic acid hematoxylin. In all autopsies performed under three hours post mortem a considerable number of the cases Zenker's, Helly's, and Champy's fluids were used for fixation, as were formalin and Regaud's method. For stains in addition to phloxine-methylene blue and phosphotungstic acid hematoxylin the granule stains of Bensley, Lane and Ullrich, Mallory's aniline blue, and Masson's three-color stains were resorted to. Serial sections were studied in some instances.

In Table 10 are seen the lesions affecting the pancreas as a whole arranged by decades.

In making the diagnosis of fibrosis only those cases showing clear-cut (though at times focal) increase in interlobular fibrous tissue with or without fibrous tissue increase in the interlobular septa were accepted and those cases which showed only slight fibrosis were omitted. In a number of instances this fibrosis was accompanied by infiltration to varying degrees with large mononuclear lymphocytes and rarely eosinophiles. In some instances entire lobules had disappeared with fibrous tissue and scattered islands and ducts remaining. The tendency of this lesion to increase in the older age group is strikingly shown being entirely absent in the 11

cases under thirty yet occurring in 36.6 per cent of the 157 cases over thirty years of age. While this frequency is markedly lower than in diabetic individuals in the same age groups it is nevertheless so great as to preclude considering it a lesion characteristic of diabetes. In nondiabetic individuals it occurs early, is frequently in association with normal islands as in those whose islands showed pathological change.

TABLE 10. LEZIONS OF THE PANCREAS IN NONDIABETICS

Age group	Cysts	Fibrosis	Lipomatosis	Acute pancreatitis	Carcinoma	Mucoid tumor	Cyst	Colests	Tuberculosis
0 to 10	1	0	0	0	0	0	0	0	0
11 to 20	2	0	0	0	0	0	0	0	0
21 to 30	3	0	0	0	0	0	0	0	0
31 to 40	10	3	0	0	0	0	0	1	0
41 to 50	34	3	5	0	0	1	0	0	0
51 to 60	63	23	10	0	1	6	1	0	0
61 to 70	57	17	8	2	0	1	0	0	1
71 to 80	24	10	4	1	0	1	1	0	0
81+	2	2	0	0	0	0	0	0	0
Total	200	58	27	3	1	14	2	1	1
Per cent of total	100	29	13.5	1.5	0.5	7.0	1.0	0.5	0.5

It seems unlikely that interacinar fibrosis is a cause of atrophy of the pancreas. Probably the fibrosis is far more frequently a result of atrophy of the parenchymatous tissue from one cause or another with resultant condensation of the stroma than an active factor in the production of pancreatic damage. The analogy to the cirrhotic process in the liver is rather striking.

In many of the cases showing fibrosis there is evidence from the anatomic findings or from the history of previous gall bladder disease. In all of these cases of fibrosis fibrous-tissue capsules can be seen about the great majority of the islands a finding which Otani considers definite evidence of a pathological process.

The criteria used for the diagnosis of lipomatosis are (1) separation of the lobules by fat cells and (2) frequent fat cells occurring among the acini. In this series the presence of lipomatosis in the pancreas was closely related to the amount of body fat. Undoubtedly the proportion of cases showing this would have been much higher were it not for the varying degrees of emaciation at times extending to almost complete depletion of the stored fat of the body in the cases of malignant disease which were fairly frequent in this series of post mortem examinations. As with fibrosis lipomatosis is associated with normal islands fully as frequently as with diseased islands.

Balog found the dry weight of the pancreas after extraction of fat to be 11 to 20 grams in the normal pancreas and 5 to 12 grams in the fatty gland.



It is rather difficult to determine whether stored fat is the only factor in producing this change or whether there is some deposit of fat in response to varying degrees of atrophy of the pancreatic parenchyma.

Primary carcinoma occurred only once—an adenocarcinoma of the head of the pancreas arising from the duct. Many of the islands persisted apparently uninjured in the midst of the tumor tissue and those islands in the uninvaded portion of the pancreas were also normal. They were not hypertrophic. This incidence of 0.5 per cent contrasts with that of 2 per cent found in our diabetic autopsy series.



FIG. 36 Carcinoma of head of pancreas, duct type. Metastases to liver. Hematoxylin counterstain.  $\times 215$ .

Metastatic tumors were frequently encountered as would be expected from the frequency of malignant disease in the post mortem material. There were 14 cases of metastatic tumor or 7 per cent. Of these 1 was malignant melanoma, 1 myelogenous leukemia, 1 lymphatic leukemia, and the remainder carcinoma. In several cases the degree of infiltration was striking but a sufficient number of islands persisted to maintain normal carbohydrate metabolism.

Tuberculosis of the pancreas was encountered once as were calculi in the pancreatic ducts. Cysts of the pancreas occurred twice, once apparently the result of a localized acute pancreatitis with subsequent atrophy and once the result of obstruction to the duct of Wirsung by fibrin of undetermined origin.

Acute pancreatitis, either hemorrhagic or gangrenous, was encountered 5 times. In four cases it was the primary cause of death and in 1 it was associated with a staphylococcus pyemia.

**Lesions of the Islands**—The occasional occurrence of lesions in the islands of nondiabetic individuals similar to those found in diabetes is a prerequisite if we consider insular changes the cause rather than the result of diabetes

Table 11 gives the frequency of lesions in the islands

TABLE 11.—LESSONS OF THE ISLANDS IN 200 NONDIABETIC PANCREASES

Age yrs	THE ISLANDS IN 200 NONDIABETIC PANCREATITIS											
	Cases	Normal	Fibrosis			Hyaline		Hypertrophy	Pyloric nuclei	Hypertrophic change	Adenoma	H morphology
			Slight	Moderate	Marked	Slight	Med rate					
0 to 10	6	0	0	0	0	0	0	0	0	0	0	0
11 to 20	12	0	0	0	0	0	0	0	0	0	0	0
21 to 30	10	0	0	0	0	0	0	0	0	0	0	0
31 to 40	10	0	0	0	0	0	0	0	0	0	0	0
41 to 50	10	0	0	0	0	0	0	0	0	0	0	0
51 to 60	17	0	0	0	0	0	0	0	0	0	0	0
61 to 70	31	0	0	0	0	0	0	0	0	0	0	0
71 to 80	54	0	0	0	0	0	0	0	0	0	0	0
81+	40	0	0	0	0	0	0	0	0	0	0	0
	23	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0
Total	200	167	10	4	1	0	3	1	5	1	2	1

able shows that practically all of the  
in diabetic individuals  
nondiabetic

This table shows that practically all of the lesions of the islands that are described in diabetic individuals are to be found although with less frequency in nondiabetic individuals. Thus, the islands were normal in 83.5 per cent of the cases. Fibrosis of the islands ranging from slight to moderate was present in 7.5 per cent. Hyalinization of the islands was present in 2 per cent. Hypertrophy of the islands in 2.5 per cent. Mitotic figures in the island epithelium in 1.5 per cent. Hypertrophic change (?) in 1 per cent. Shrunken cells with pyknotic nuclei in 1 per cent. and hemorrhage in the islands and an adenoma of the islands may be brought about by the same causes that produce the interisular fibrosis. This latter lesion appeared in 10 out of 15 pancreases that showed fibrosis in our first series.

Hyalinization of the islands has been considered perhaps the most characteristic lesion of diabetes. That the occurrence of this hyalinization was not a sufficient ground for establishing a diagnosis of diabetes pathologically was shown by the case of Ohlmer<sup>4</sup> who reported the occurrence of hyaline islands in the pancreas of a nondiabetic aged seventy-two years under observation for nine months previous to death. Four fifths of the islands showed hyaline degeneration but many of the surviving ones were hypertrophied which probably accounts for the absence of diabetic symptoms. Saltykow<sup>5</sup> reported hyaline degeneration of some of the islands of Langerhans in the pancreas of a male aged eighty-eight years with advanced arteriosclerosis. Cecil<sup>6</sup> in a careful study of these lesions in nondiabetics

It is rather difficult to determine whether stored fat is the only factor in producing this change or whether there is some deposit of fat in response to varying degrees of atrophy of the pancreatic parenchyma.

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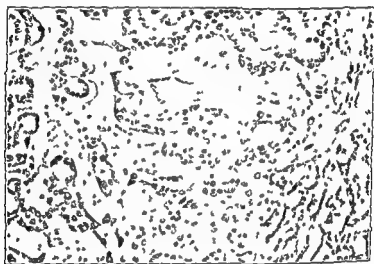


FIG. 36.—Carcinoma of head of pancreas, duct type. Metastasis to liver. Hematoxylin-eosin stain.  $\times 215$ .

Metastatic tumors were frequently encountered as would be expected from the frequency of malignant disease in the post mortem material. There were 14 cases of metastatic tumor or 7 per cent. Of these 1 was malignant melanoma, 1 myelogenous leukemia, 1 lymphatic leukemia, and the remainder carcinoma. In several cases the degree of infiltration was striking but a sufficient number of islands persisted to maintain normal carbohydrate metabolism.

Tuberculosis of the pancreas is apparently the result of infection, but in some cases it is the result of obstruction to the duct of Wirsung by a tumor of undetermined origin.

Acute pancreatitis, either hemorrhagic or gangrenous, was encountered 5 times. In four cases it was the primary cause of death and in 1 it was associated with a staphylococcus pneumonia.

**Lesions of the Islands.**—The occasional occurrence of lesions in the islands of nondiabetic individuals similar to those found in diabetes is a prerequisite if we consider insular changes the cause rather than the result of diabetes.

Table 11 gives the frequency of lesions in the islands.

TABLE 11.—LESSONS OF THE ISLANDS IN 200 NONDIABETIC PANCREASES

Age yrs	Fibrosis									
	Case	Normal	Slight	Moderate	Marked	Atroph	Atroph	Hypertrophy	Pyknotic nuclei	Hypertrophy
0 to 10	0	0	0	0	0	0	0	0	0	0
11 to 20	3	0	0	0	0	0	0	0	0	0
21 to 30	10	0	0	0	0	0	0	0	0	0
31 to 40	10	0	0	0	0	0	0	0	0	0
41 to 50	74	0	0	0	0	0	0	0	0	0
51 to 60	61	0	0	0	0	0	0	0	0	0
61 to 70	54	0	0	0	0	0	0	0	0	0
71 to 80	40	0	0	0	0	0	0	0	0	0
81+	12	0	0	0	0	0	0	0	0	0
Total	167	10	4	1	0	1	1	5	2	1

This table shows that practically all of the lesions of the islands that are described in diabetic individuals are to be found although with less frequency in nondiabetic individuals. Thus the islands were normal in 83.5 per cent of the cases. Fibrosis of the islands ranging from slight to moderate was present in 7.0 per cent. Hyalinization of the islands was present in 2 per cent. Hypertrophy of the islands in 2.5 per cent. Mitotic figures in the island epithelium in 1.5 per cent. Hydropic change (?) in 1 per cent. Shrunken cells with pyknotic nuclei in 1 per cent. and hemorrhage in the islands and an adenoma of island type each in 0.5 per cent.

Presumably fibrosis of the islands may be brought about by the same causes that produce the interisular fibrosis. This latter lesion appeared in 10 out of 15 pancreases that showed fibrosis of the islands. This is not quite so high a proportion as was noted in our first series.

Hyalinization of the islands has been considered perhaps the most characteristic lesion of diabetes. That the occurrence of this hyalinization was not safe ground for establishing a diagnosis of diabetes appeared in the case of Ohlmacher<sup>4</sup> who reported the occurrence of hyaline islands in the pancreas of a nondiabetic aged seventy-two years under observation for nine months previous to death. Four fifths of the islands showed hyaline degeneration but many of the remaining ones were hypertrophied which probably accounts for the absence of diabetic symptoms. Saltzko<sup>5</sup> reported hyaline degeneration of some of the islands of Langerhans in the pancreas of a male aged eighty-eight years with advanced arteriosclerosis. Cecil<sup>6</sup> in a careful study of these lesions in nondiabetics

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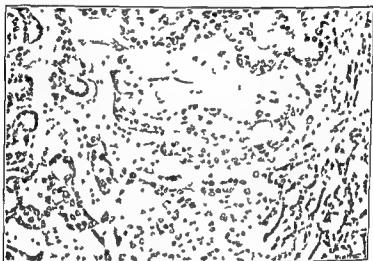


FIG. 30 — Carcinoma of head of pancreas duct type. Metastasis to liver. Hematoxylin-eosin stain.  $\times 215$ .

Metastatic tumors were frequently encountered as would be expected from the frequency of malignant disease in the post mortem material. There were 14 cases of metastatic tumor or 7 per cent. Of these 1 was malignant melanoma, 1 myelogenous leukemia, 1 lymphatic leukemia, and the remainder carcinoma. In several cases the degree of infiltration was striking but a sufficient number of islands persisted to maintain normal carbohydrate metabolism.

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Acute pancreatitis, either hemorrhagic or gangrenous, was encountered 5 times. In four cases it was the primary cause of death and in 1 it was associated with a staphylococcus pyemia.

# LESIONS OF PANCREAS IN NON DIABETIC INDIVIDUALS 79

**Lesions of the Islands**—The occasional occurrence of lesions in the islands of nondiabetic individuals similar to those found in diabetes is a prerequisite if we consider insular changes the cause rather than the result of diabetes

Table 11 gives the frequency of lesions in the islands

TABLE 11—LESIONS OF THE ISLANDS IN 200 NONDIABETIC PANCREASES

Age yrs	Fibrosis											
	Chronic				Hyaline							
	Chronic	Normal	Slight	Marked	No lesion	Marked	Slight	Marked	Hypertrophy	Atrophy	Islet not evident	Adenoma
0 to 10	0	0	0	0	0	0	0	0	0	0	0	0
11 to 20	0	0	0	0	0	0	0	0	0	0	0	0
21 to 30	0	0	0	0	0	0	0	0	0	0	0	0
31 to 40	0	0	0	0	0	0	0	0	0	0	0	0
41 to 50	0	0	0	0	0	0	0	0	0	0	0	0
51 to 60	0	0	0	0	0	0	0	0	0	0	0	0
61 to 70	0	0	0	0	0	0	0	0	0	0	0	0
71 to 80	0	0	0	0	0	0	0	0	0	0	0	0
81+	0	0	0	0	0	0	0	0	0	0	0	0
Total	167	10	4	1	0	1	5	1	1	1	1	1

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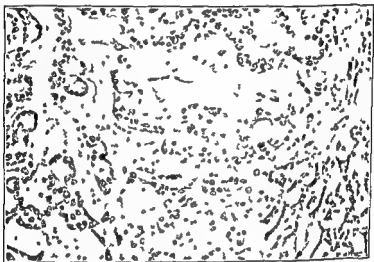


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Metastatic tumors were frequently encountered as would be expected from the frequency of malignant disease in the post mortem material. There were 14 cases of metastatic tumor or 7 per cent. Of these 1 was malignant melanoma, 1 myelogenous leukemia, 1 lymphatic leukemia, and the remainder carcinoma. In several cases the degree of infiltration was striking but a sufficient number of islands persisted to maintain normal carbohydrate metabolism.

Tuberculosis of the pancreas was encountered once as were calculi in the pancreatic ducts. Cysts of the pancreas occurred twice, once apparently the result of a localized acute pancreatitis with subsequent autolysis and once the result of obstruction to the duct of Wirsung by fibrosis of undetermined origin.

Acute pancreatitis, either hemorrhagic or gangrenous, was encountered 5 times. In four cases it was the primary cause of death and in 1 it was associated with a staphylococcus pyemia.

# LESIONS OF PANCREAS IN NON DIABETIC INDIVIDUALS 81

uals is similar to that occurring in the islands of diabetic individuals. Stains for glycogen were not used.

Again hemorrhage in the island is probably an artifact or at least in agonal change. This lesion is encountered once in the present series. The pancreatic tissue is fairly unsuceptible so far as actual necrosis is concerned to injury from toxic causes as compared with such tissues as liver or kidney, and so regenerative changes are rarely seen. This probably accounts for the rarity of diabetes after infections. Mitoses are occasionally encountered in the island cells (3 times in this series) but are more frequently than would be expected in slow replacement of worn-out cells.

The curious epidermoidization of the ducts to which attention has been called by Baló and Ballón in "Praxis" Lotun in 1912 and others has been found only once in this series.

**Summary**—It is striking that practically any lesion found in the pancreas of diabetic individuals either involving the island tissue or the acinar tissue or both can be duplicated in the pancreas of nondiabetics. However the frequency of lesions of the island is much less in nondiabetic individuals than in diabetics. Therefore the occurrence of any of the generally accepted insular lesions may be considered as presumptive evidence of diabetes. However without studies of the glycogen distribution in other organs and perhaps differential counts on the islands as representation of diabetes cannot be made. Interstitial pancreatitis as represented by fibro is occurs two frequently and in too wide a range of disease to be considered a characteristic lesion of diabetes. Lipomatosis is probably more nearly related to the amount of stored fat in the body than it is to pathological changes in the pancreas.

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reports 3 cases of his own and one of Whipple's. In addition he states that Milne and Peters<sup>7</sup> mentioned hyaline degeneration of the islands in non-diabetic conditions.

Wright<sup>8</sup> has reported 5 cases all over fifty years of age showing varying degrees of hyalinization of the islands associated with slight to marked intercapillary fibrosis and showing slight to moderate arteriosclerosis. In this series 3 cases of moderate hyalinization of the islands were encountered and one of marked hyalinization all over forty years of age. The youngest case one of Cecil's was thirty-seven years of age. Arrangement and staining properties of the hyaline in nondiabetics are identical with those of the islands of diabetic individuals.

The staining reactions of amyloid may also be found in the hyaline of nondiabetic islands. Aron<sup>9</sup> found hyaline which he regarded as amyloid in 16.6 per cent of his nondiabetic series and Ahronheim<sup>10</sup> obtained positive reactions for amyloid in 5 of 50 nondiabetics.

Lymphocytic infiltration of the islands was found by Susman<sup>11</sup> in a non-diabetic woman aged forty-six years who died of Addison's disease secondary to adrenal atrophy.

Hypertrophy of the islands was encountered in 5 cases and 4 of these showed fairly marked interstitial pancreatitis. There is some discrepancy in statements as to the normal size of the islands. However a diameter of 400 micra has been considered the upper normal limit by Cecil<sup>1</sup> and this has been used as the criterion in the present study.

There is one difference however between the hypertrophied islands of the diabetic and the nondiabetic. Frequently the hypertrophied islands of a diabetic individual consist of elongate cells with narrow centrally located nuclei whereas the hypertrophied islands in the pancreas of non-diabetic individuals are made up of the usual polyhedral cells with rounded or slightly oval nuclei.

In relation to the work of Ferner cited in Chapter one wonder whether this difference may not be due at least in some cases to a predominance of  $\alpha$  cells in the hypertrophied island of the diabetic.

Adenoma of island-cell type is a rare lesion. In 1926 one of us (S. W.) reported<sup>12</sup> 4 cases and collected 16 others from the literature. In only one case that of Heiberg<sup>13</sup> was diabetes present. In the present series one case was found occurring in a female aged forty-four years dying from epidermoid carcinoma of the cervix uteri. Further discussion of insular adenomas may be found in Chapter 22.

It is probable that the occurrence of pyknotic nuclei in the island cells is an artifact at least in nondiabetics but as this has been considered as a possible diabetic lesion (Gibb and Logan<sup>14</sup>) the occurrence has been recorded in Table 11.

Similarly hydropic change at least so far as it occurs in nondiabetics is probably due to post mortem change. However its appearance in the 2 instances in which it occurred in the present series of nondiabetic in island

(Bernick *et al.*) showed that the conversion of  $^{14}$ C-labeled glucose to fatty acids is drastically reduced in liver slices of alloxan-diabetic rats confirming Stetten's conclusion that the failure to utilize glucose for fatty acid synthesis is a major metabolic defect in diabetes. They also found that insulin would completely repair this defect.

It has also been shown<sup>10</sup> that the liver is the site of the conversion of glucose to long-chain fatty acids (as well as short chain) and that in diabetic rats these same fatty acids are readily converted back to glucose. Hastings and his co-workers<sup>11-13</sup> have studied various aspects of the synthesis of glycogen by liver slices *in vitro* including the incorporation of  $^{14}$ C derivation of glycogen from acetate and pyruvate effects of the ionic environment, etc.

Hinkoff<sup>14</sup> has summarized some of the newer contributions in this field.

## GLYCOGEN DISTRIBUTION

Although diabetes mellitus is primarily a disease of carbohydrate metabolism we know very little from the histological standpoint regarding it. The solubility of the various sugars in the body fluids makes it practically impossible to utilize any of the ordinary histological procedures in study of the problem. The carbohydrate that we can study most readily by histologic means, glycogen, is fortunately of great importance and there are abnormalities of glycogen distribution that characterize diabetes. Some of these changes, such as the glycogenic infiltration of the renal tubular epithelium were recognized long before their nature was understood. Thus Armannus<sup>15</sup> described a hyaline vacuolization of the epithelium of Henle loops in mice that it was due to the presence of glycogen within the epithelial cells.

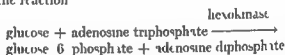
Sumner<sup>16</sup> and Hinkoff<sup>17</sup> in 1938, found in 14 cases of diabetes this peculiar infiltration of the epithelium of Henle's loops in the kidneys to be due to the presence of glycogen. This lesion has been regarded as the most characteristic anatomical finding in active diabetes mellitus. Other less constant disorders of glycogen deposits associated with the disease were also noted by Hinkoff. The increased glycogen in the heart muscle fibers, glycogenic granules in the leukocytes and large glycogen filled vacuoles in the liver cells, all followed by Best<sup>18</sup> who demonstrated the glycogen filled vacuoles in the epithelial cells of the liver to be the nucleus.

**Methods.** While Best's carmalum stain is still a useful method, the best available technique appears to be the periodic acid-Schiff reaction method for staining fixation in chilled Rossman's fluid. Alcohol fixation is likewise an essential good results being obtained after formalin fixation is likewise shown in paraffin sections with various stains the presence of vacuolization runs closely parallel with the glycogen content as shown by Best's

## Chapter 5

### PATHOLOGICAL EVIDENCES OF ABNORMAL CARBOHYDRATE METABOLISM

RECENT advances in the physiology of the metabolism of carbohydrates have greatly exceeded those in the histological distribution and histochemistry of these substances. Stetten<sup>1</sup> has recently summarized modern concepts of the metabolism of carbohydrates. He emphasizes among other points that one may no longer speak of simple oxidation or burning of carbohydrate and that the boundary lines between the metabolism of protein, fat, and carbohydrate are no longer distinct, since it has been shown that their catabolic pathways cross at many points and that each of these three major nutrients may supply organic fragments which feed into the tricarboxylic acid cycle. He also indicates the fundamental importance of the reaction



especially in view of the fact that the anterior pituitary seems to produce a specific inhibitor of the ubiquitous enzyme hexokinase (although it seems to affect the hexokinase of muscle and liver more than that of other cell types) and that insulin seems to act to antagonize or neutralize this inhibitor.

Much light is being shed on problems of carbohydrate and fat metabolism by the use of radioactive carbon ( $C^{14}$ ). Thus Stetten<sup>1</sup> showed that of 30 gm. of carbohydrate given to the normal rat only 1 gm. is stored as glycogen, 10 gm. are stored as fat, and 19 gm. are broken down into the metabolic pool for energy and for conversion to protein, fat, or other substances. Zilverman *et al.*<sup>2</sup> found that the over-all rate of oxidation of glucose by the alloxan-diabetic rat did not differ significantly from that of the normal. Viles and Hastings<sup>3</sup> using the isolated rat diaphragm technique showed that when no insulin was added glucose utilization and glucose metabolized to carbon dioxide were lower than normal in muscle from alloxan-diabetic rats and greater than normal in muscle from adrenalectomized, hypophysectomized, and adrenalectomized hypophysectomized animals, also that glucose synthesis without insulin was lower than normal in diabetic and adrenalectomized rats and greater in hypophysectomized, diabetic, adrenalectomized, and hypophysectomized adrenalectomized rats. The addition of insulin increased the amount of glucose appearing as  $CO_2$  in all but the diabetic diaphragm, in which the increase was not significant.

heart, liver, kidneys, pancreas, voluntary muscle and skin. Both insulin treated cases of diabetes and cases not treated with insulin are included as well as a control series of nondiabetics.

**Heart** Glycogenic infiltration of the heart muscle is marked in many cases of diabetes. It is particularly striking in those fibers at the margins of infarcts. It can also be found there longer post mortem. Whether this means greater storage of glycogen or that the fibers are damaged and so deficient in glycogenolytic ferment is somewhat difficult to decide. On the basis of tissue fixed under thirty minutes post mortem, an abnormally large storage of glycogen seems the more likely.

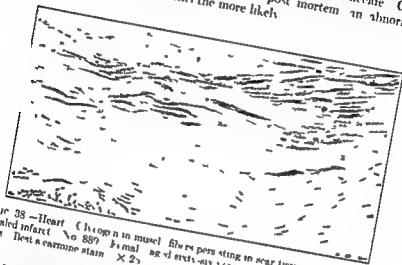


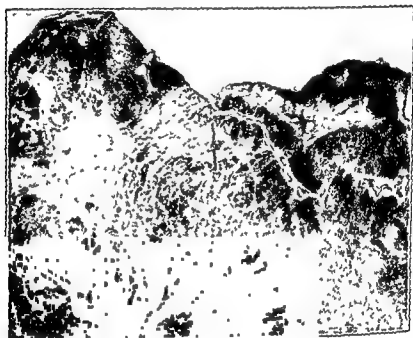
Fig. 38.—Heart. Glycogen in muscle fibers persisting in scar tissue at the margin of a healed infarct. No. 8897. Normal age of infarct—six years. Duration of diabetes fifty years. Best a carmine stain.  $\times 27$ .

So far as the amount of glycogen in the muscle as a whole is concerned there is but little difference between the insulin treated cases of diabetes and the control series. The diabetic cases not treated with insulin show a high proportion of heavy deposits of glycogen.

Cruckshank<sup>10</sup> has made an extensive study of cardiac glycogen showing that it is definitely and characteristically increased in both human and experimental diabetes. He has also pointed out the reasons for its deposition in this site and the unexplained fact that the amount of glycogen is about 100% greater than the normal, i.e., that the heart has lost its power to store glycogen.

stain or the periodic acid method in tissues fixed under one hour post mortem, and is much more marked than the stain would indicate in tissues fixed a longer time post mortem

In most tissues there is a rapid drop in demonstrable glycogen in the first hour, and but little change for some hours thereafter. Yater, Osterberg, and Hefke<sup>18</sup> found by microchemical analysis that one-half to three-fourths of the glycogen in the rabbit disappears in the first hour and that from then on the rate of decrease is slow



Once fixation has occurred, change in glycogen is slight or none. But little change is detected in staining property of glycogen in absolute alcohol-fixed tissue after ten years. Brann<sup>19</sup> found 3 per cent glycogen present in a liver Kaiserling-preserved for eighteen months. Ordinary formalin will inhibit enzyme action and so prevent glycogenesis. Traces of both intracellular and intranuclear glycogen have been demonstrated in this laboratory in formalin-fixed diabetic livers after preservation for over twelve years.

Old paraffin-embedded tissue is quite suitable for glycogen stains, even though the original fixative may have been in aqueous solution.<sup>14</sup>

**Results.**—Both surgical and post-mortem material has been utilized, and the glycogen distribution studied in the various tissues, particularly



FIG. 41—Moderate amount of glycogen in cells of periportal zone of liver. No glycogen in liver cell nuclei. The effect of insulin treatment. D127-34. Female aged seventy-three years. Duration of diabetes nine years. Best's carmalum stain  $\times 95$ .

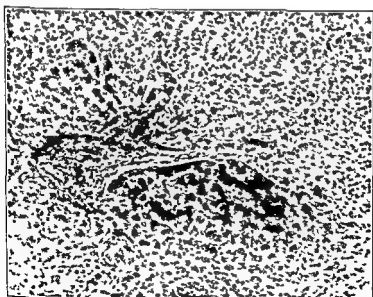


FIG. 42—Slight amount of glycogen restricted to liver cells of periportal zones. No glycogen in liver cell nuclei. The effect of insulin treatment. D127-34. Female aged sixty-seven years. Duration of diabetes two years. Best's carmalum stain  $\times 95$ .



FIG. 31.—Glycogenic infiltration (dark masses) in liver cell nuclei. Note very slight amount of glycogen in liver cells. Fifty minutes post mortem. D427-66. Male aged sixty six years. Duration of diabetes seven months.  $\times 150$

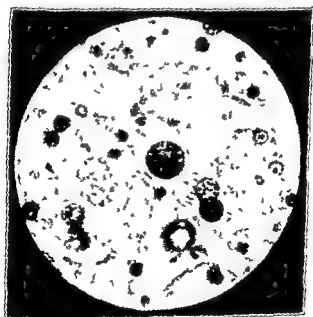


FIG. 40.—Glycogenic infiltration of liver cell nuclei. Liver cells devoid of glycogen. Rest's carmine stain. A22-44. Male aged seventy four years. Duration of diabetes eight years.  $\times 500$  (Joshin)

not only on glycogen formation by the nucleus but also on the permeability of the nuclear membrane for glycogen.

TABLE 12.—GLYCOGEN CONTENT OF LIVER-CELL NUCLEI

Type of treatment	None		Slight		Moderate		Marked	
	No.	%	No.	%	No.	%	No.	%
No insulin	8	21	13	34	8	21	1	24
Insulin	28	6	11	26	3	—	1	2
Insulin + fraction present	6	5*	13	19	18	18	6	6
Control (nondiabetics)	16	5	3	31	3	10	1	4

Glycogen is most marked in those cells in the periportal region. Frequently the cells about the central vein contain none although it is present elsewhere in the lobule in large amounts.

There is no antagonism between the deposition of fat and glycogen both being often present in the same cells in large amounts. The histological foundation for the rather prevalent belief in this antagonism is probably the tendency of glycogen to be deposited at the periphery of the lobule first and fat to be most prominent about the central vein. However these dis-

rendered invisible in these cells with an adequate blood supply where is the cells about the central veins cells which have to sit at second table may be fairly easily loaded with fat which they cannot metabolize and which therefore remains in visible form within the cells.

For some reason the rabbit, in contrast to the human, shows the glycogen deposits to be chiefly in the central portion of the lobule.<sup>22, 23</sup> In the dog an even distribution through the lobule is the rule but in hepatic injury the deposition is most marked about the central veins.<sup>24</sup>

Peculiarly enough liver glycogen seems to be increased in the alloxan diabetic rat.<sup>25, 26</sup> The explanation is obscure. Overactivity of the adrenal cortex may be partly responsible.<sup>27</sup>

Estimations of liver glycogen vary greatly. Ippinger<sup>28</sup> gives the possible amount of glycogen in the liver of a well nourished man as 400 grams (20 to 25 per cent). Dudley and Murrin<sup>29</sup> found 5.5 per cent in rabbits and Fisher and Lackey<sup>30, 31</sup> 4.6 per cent in fed and 0.3 per cent in fasting dogs.

TABLE 13.—GLYCOGEN CONTENT OF LIVER CELLS

Type of treatment	None		Slight		Moderate		Marked	
	No.	%	No.	%	No.	%	No.	%
No insulin	9	29	11	35	8	26	3	10
Insulin	—	—	8	24	12	36	13	40
Insulin + fraction present	—	8	26	31	32	38	20	23
Control (nondiabetics)	1	3	10	35	14	48	4	14



It seems probable that the hearts of infants of diabetic mothers also tend to have a higher than normal content of glycogen (see Table 41, page 252).

*Liver* Since glycogenic distention of the nuclei of liver cells is found in many other conditions than diabetes, this cannot be regarded as of particular importance. However, in diabetic patients there is a very definite reciprocal relationship between the amount of intranuclear and the amount of intracytoplasmic glycogen. When intranuclear glycogen deposition is marked there is but little or even no glycogen in the liver cells in these cases. Conversely, when there is much glycogen stored in the cytoplasm, there are few or no nuclei containing it.



FIG. 43.—Liver showing no  
to periportal zone. No glycogen.  
1020 Male aged fifty years  
stain  $\times 25$

regard the vacuolation as a reliable index of glycogen.

Eger and Klarner<sup>21</sup> maintain that glycogen is *formed* in the nucleus of the liver cell and then deposited or stored in the cytoplasm. The accumulation in the nucleus in diabetes is regarded as indicating that insulin has an effect

Hiddle *et al.*,<sup>11</sup> studied a number of normal controls as well as diabetics using both chemical and histological (Best's (armine) methods. In liver biopsies they found variations in 19 normals from 0.9 to 4.1 per cent with a mean of 2.15 per cent and in 10 diabetics from 0.88 to 2.98 per cent with a mean of 1.84 per cent. In muscle biopsies (pectoralis major) they found 15 normals ranging from 1.13 to 3.89 per cent with a mean of 2.20 per cent and 6 diabetics from 0.72 to 2.49 per cent with a mean of 1.59 per cent. They did not consider the slightly lower values in the diabetics as significant. They found no correlation of the glycogen values with type of diabetes, fasting blood sugar level or degree of ketosis.

**Pancreas.** In 8 instances considerable amounts of glycogen and in 12 instances slight amounts were found in the epithelium of the pancreatic ducts both the smaller ducts with cuboidal epithelium and the larger ducts with columnar. Ohlsson<sup>12</sup> found this distribution in mammalian species. From the recent work of Toreson<sup>13</sup> cited more fully in Chapters 3 and 27 it appears that glycogen may be found in the  $\beta$  cells of the islets (as well as in the ducts) in diabetes and may be associated with hydropic changes which he prefers to call glycogen infiltration. Further work will be required to decide how frequently glycogen is to be found in this situation. One wonders whether it indicates cell injury or represents a storage phenomenon as in other tissues. Duff and Larsson<sup>14</sup> were able to prevent or reverse "hydropic change" in alloxan-diabetic rabbits with small doses of insulin in spite of continued hyperglycemia. They suggest that hyperinsulinemia is more important in the pathogenesis of this lesion than is hyperglycemia. Toreson<sup>13</sup> makes the interesting suggestion that it may be a sign of regeneration thus correlating with the glycogen in the ducts from which new strands are usually formed. Glycogen infiltration of the islets can be produced in rabbits with large doses of cortisone (Figs. 13 and 14).

TABLE 11. EFFECT OF INSULIN TREATMENT ON GLYCOGEN DISTRIBUTION

Type of treatment	No. of animals	% of glycogen in islets	% of glycogen in ducts	% of glycogen in tubules	% of glycogen in total
Insulin	4	2	1	5	2
Insulin + cortisone	5	100	1	5	2
Insulin + cortisone	11	5	1	5	2
Insulin + cortisone	2	100	1	5	2

**Kidneys.** Insulin treatment definitely reduces the amount of glycogen present in the kidneys as Root and Warren<sup>15</sup> pointed out. In many cases glycogen is entirely absent. In many others it is considerably reduced. That found in cells of diabetics not treated with insulin. Although Henkels<sup>16</sup> has in the most typical case for glycogenic infiltration in the tubules as well as occasionally involved. This latter infiltration is sometimes named after Flatau<sup>17</sup> who noted the deposits but considered them to be mere debris of the cells of the tubules. Glycogenic granules may even

feeding dogs a diet similar to Port's standard diet for mice Junkers<sup>10</sup> obtained a value of 6.1 per cent. A maximum of 4.4 per cent was attained by Selter's mice.

The above table gives evidence of the effect of insulin in promoting the deposit of glycogen in the liver although this effect is somewhat lessened in septic cases. In severe diabetics without insulin even chemical examination may show no or very little glycogen in the liver.<sup>11</sup> The one nondiabetic liver showing no glycogen histologically was a case of healed toxic sclerosis with jaundice. What bearing this may have on the clinical finding that

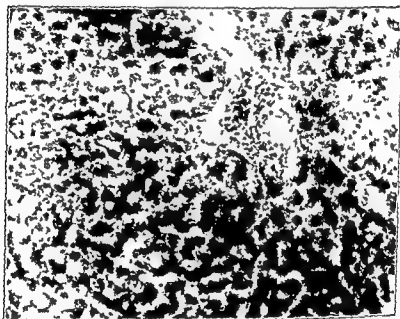


Fig. 44. Liver showing marked glycogenic infiltration of liver cells. Effect of insulin treatment. Absence of fatty infiltration. D4977. 1 ml. of glucose 10% solution. Duration of diabetes 12 years. Best specimen.  $\times 150$ .

cases of cirrhosis of the liver and diabetes do not do so well under insulin treatment as other cases is uncertain but it suggests that there may be impairment of carbohydrate storage in cirrhosis. Ravid<sup>12</sup> has summed up the evidence in favor of this view and concludes that injured liver cells have decreased ability to synthesize and store glycogen.

Recently a few studies of liver glycogen have been made in the living patient by needle biopsy. Bondy and Sheldon<sup>13</sup> in a case of diabetic coma which had been untreated for ten hours found only a trace of glycogen at a liver biopsy. After seven and one-half hours of treatment there was marked restoration of glycogen but fat vacuoles were numerous. Six days later there was a slight further increase in glycogen but no change in the fat

Hildes *et al.*<sup>24</sup> studied a number of normal controls as well as diabetics using both chemical and histological (Best's carmine) methods. In liver biopsies they found variations in 19 normals from 0.95 to 4.1 per cent with a mean of 2.15 per cent, and in 10 diabetics from 0.88 to 2.98 per cent with a mean of 1.84 per cent. In muscle biopsies (pectorals major) they found 15 normals ranging from 1.13 to 3.89 per cent with a mean of 2.20 per cent and 6 diabetics from 0.72 to 2.49 per cent with a mean of 1.59 per cent. They did not consider the slightly lower values in the diabetics as significant. They found no correlation of the glycogen values with type of diabetes, fasting blood sugar level, or degree of ketosis.

**Pancreas.** In 8 instances considerable amounts of glycogen and in 12 instances slight amounts were found in the epithelium of the pancreatic ducts, both the smaller ducts with cuboidal epithelium and the larger ducts with columnar. Ohohishi<sup>25</sup> found this distribution in mammalian fetuses.

From the recent work of Toreson<sup>15</sup> cited more fully in (Chapter 3) and 25 it appears that glycogen may be found in the  $\beta$  cells of the islands (is well as in the ducts) in diabetes and may be associated with 'hydropic change' which he prefers to call glycogen infiltration. Further work will be required to decide how frequently glycogen is to be found in this situation. One wonders whether it indicates cell injury or represents a storage phenomenon as in other tissues. Duff and Forson<sup>26</sup> were able to prevent or reverse 'hydropic change' in alloxan-diabetic rabbits with small doses of insulin in spite of continued hyperglycemia. They suggest that hypoinsulinemia is more important in the pathogenesis of this lesion than is hyperglycemia. Toreson<sup>15</sup> makes the interesting suggestion that it may be a sign of regeneration, thus correlating with the glycogen in the ducts from which new islands are usually formed. Glycogen infiltration of the islands can be produced in rabbits with large doses of cortisone (Figs. 13 and 14).

TABLE 14. GLYCOGEN CONTENT OF PANCREATIC DUCTS

Type of treatment	Area		No./g.		Mod. #		Mean	
	No.	%	No.	%	No.	%	No.	%
No insulin	4	27	6	40	3	20	2	11
Insulin	5	100						
Insulin + fraction present	11	53						
Control (non-diabetics)	25	100	6	30	3	15		

**Kidney.** Insulin treatment definitely reduces the amount of glycogen present in the kidneys, as Root and Warren<sup>27</sup> pointed out. In many cases glycogen is entirely absent. In many others it is considerably reduced over that found in cases of diabetes not treated with insulin. Although Henks<sup>28</sup> loops are the most typical loci for glycogenic infiltration, the convoluted tubules as well are occasionally involved. This latter localization is sometimes named after Lieberkuhn<sup>29</sup> who noted the deposits but considered them to be necrosis of the cells of the tubules. Glycogenic granules may even

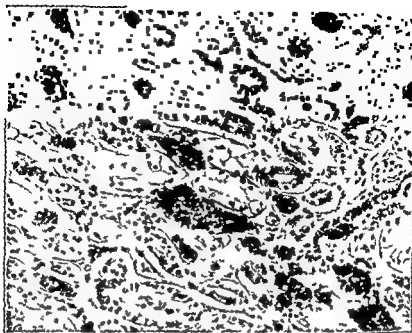


FIG. 45.—Kidney showing marked glycogenic vacuolization of epithelium of convoluted tubules so-called Hirst's degeneration. No. 5151. Female aged fifty-six years. Duration of diabetes ten years. Phloxalungst and hematein stain.  $\times 225$ .



FIG. 46.—Kidney showing marked glycogen deposition in epithelium of Henle's loop. A22-44. Male aged seventy-four years. Duration of diabetes eight years. Best's carmine stain.  $\times 560$ .

TABLE 15—GLYCOGEN CONTENT OF KIDNEYS

Type of treatment	None		Slight		Moderate		Marked	
	No.	%	No.	%	No.	%	No.	%
No insulin	6	12	3	16	10	20	26	02
Insulin infection present	15	35	15	35	9	21	4	1
Control (nondiabetic)	21	23	28	27	19	17	13	11
	18	9	1	5				

Practically every case of active diabetes either untreated or treated in the pre-insulin days showed glycogenic infiltration of the epithelium of Henle's loops at autopsy. Its absence in many cases treated with insulin shows that it is not an essential lesion of diabetes. This is also indicated by its presence in phloridzin diabetes. Thus von Clerke<sup>22</sup> states it has been found

- 1 In diabetes mellitus
- 2 After experimental extirpation of the pancreas
- 3 After extirpation of the renal plexus
- 4 In phloridzin poisoning

In the first three instances the blood sugar level is increased in the first 3 normal or low. The one point in common is the presence of glucose since in the first 3 instances of excess glycosuria in the human material the presence of glycogen in Henle's loops may for all practical purposes be interpreted as an indication of active diabetes. It is rather interesting that Ohkashy<sup>23</sup> found that the distribution of glycogen in the kidney of the diabetic human is exactly the same as that in the normal kidney of the Anuro (See also page 168). Hudec<sup>24</sup> has demonstrated glycogen in the collecting tubules of the kidney in the newborn of four species (rabbit guinea pig rat and man). Loewen<sup>25</sup> gives the following table based on 6 cases of diabetes showing the glycogen present elsewhere in the kidneys besides Henle's loops

TABLE 16—LOCATION OF GLYCOGEN IN RENAL TUBULES (Loewen)

Case	Distal tubules	Control dist tubules	Henle's loops
I	+++	+++	++
II	++	++	++
III	+	+	+++
IV	-	-	++
V	-	-	+++
VI	-	-	+

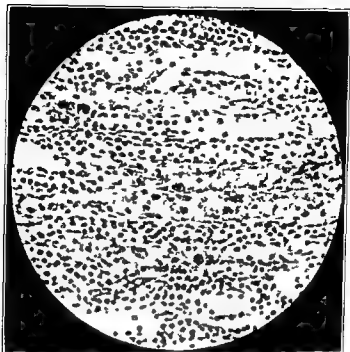


Fig. 47.—Renal glomeruli of the kidney showing vacuolization and glycogen granules. M886. Male, aged fifty-four years. Duration of diabetes thirty years. Best's carmalum stain.  $\times 170$ .



Fig. 48.—Glycogenic infiltration of epithelium of convoluted tubules. No. 5153. Female, aged fifty-six years. Duration of diabetes ten years. Best's carmalum stain.  $\times 150$ .

Rosenberg<sup>10</sup> also mentions a case in which glycogen was present in the glomeruli and the epithelium of the convoluted tubules as well as in Henle's loops. Certain of the cases summarized in this series have shown a similar distribution of the glycogen. Sandmeier<sup>11</sup> gives a quantitative determination of the glycogen present in the kidney of a girl aged nine years who had had diabetes for two years. 0.1155 gram per 100 grams of kidney substance. The liver contained 0.6130 gram per 100 grams or nearly six times as much.

There is close correspondence between increase in liver-cell glycogen and decrease in renal glycogen and a similar relationship between liver-cell glycogen and liver nucleus glycogen. Thus as normal storage increases under the influence of insulin pathological storage decreases.<sup>12</sup> This holds true in other organs as well. A diabetic responding satisfactorily to insulin treatment and dying of some condition other than diabetes or sepsis such as cerebral hemorrhage shows very nearly the same distribution of glycogen as does the normal individual. In some cases it has been possible to correlate with the glycogen deposits in kidney and liver the blood and urine sugar levels obtained within a few hours before death. Typical results are shown in Table 17.

TABLE 17.—RELATION OF GLYCOGEN IN BLOOD AND URINE TO URINE SUGAR

Case No.	Insulin	Blood sugar mg per cent	Urine sugar mg per cent	Liver glycogen		Kidney glycogen	
				Cells	% of cells	Henle's loops	Corrosion table
D426-5 <sub>1</sub>	+	410	0.2	+	+	+	+
D42 <sup>12</sup> -12	+	160	1.1	++	+	+	+
D42 <sup>12</sup> -13	+	50	5.5	+++	+	+	+
D42 <sup>12</sup> -11	+	600	1.0	+++	+	+	+
881	+	180	0.0	+++	+	+	+
1603	+	730	0.8	+	+	+	+
709	+	260	0.1	+++	+	+	+
5142	+	110	6.2	++	++	++	++
5461	+	720	2.5	+	++	++	++
517	+			+	++	++	++

There is a fair degree of correspondence between the urine sugar and the amount of glycogenic infiltration of the kidney, considering that variations in urine sugar must occur rather more rapidly than variations in glycogen content of the renal epithelium.

These results have received experimental confirmation in the work of Curtis<sup>13</sup> and Richblum.<sup>14</sup> They studied the glycogen content of the renal tubules in a group of rats made diabetic with alloxan. They found that the appearance of glycogen in the renal tubules depended solely upon the terminal blood sugar levels of the animals. A value of 150 mg per cent was the critical level above which glycogen was almost invariably de-



monstrable. With terminal blood sugar levels below 300 mg per cent no glycogen was found in the kidneys.

**Voluntary muscles.** Many specimens of muscle came from legs amputated for gangrene. They therefore may possibly be of dubious value owing to the influence of an impaired circulation which would certainly interfere with normal cellular function and might well influence the function of glycogen storage. However, the muscle was always taken from as near the site of amputation as was possible without obtaining traumatized tissue. In this way healthy tissue could be obtained. Thanks are due to Dr L. S. McKittick for permitting the use of fresh surgical material from his cases. In the autopsy material portions of the pectoralis major were utilized.

TABLE 18 — GLYCOGEN CONTENT OF VOLUNTARY MUSCLE

Type of treatment	None		Slight		Moderate		Marked	
	No.	%	No.	%	No.	%	No.	%
No insulin			1	14	5	22	1	14
Insulin	1	8	3	27	9	60		
Insulin infection present	1	1	29	39	30	40	15	20
Control (nondiabetics)			"	20	12	44	8	30

Although the results summarized in Table 18 are perhaps not statistically significant there is a tendency for decrease in muscle glycogen in the cases of diabetes as would be expected from our knowledge of the disease.

Muscle glycogen has not so great significance for the diabetic as liver glycogen since only liver glycogen is directly transformable into glucose available for use of all the cells of the body.<sup>47</sup>

Sim Unna<sup>48</sup> has called attention to the presence of glycogen in normal skin. Recent work suggests that relatively little is present under usual circumstances.

When it is present the epithelium of the stratum corneum, sweat glands, sebaceous glands, and hair follicles contains most. Becker<sup>49</sup> speaks of much glycogen in the duct cells of sweat glands in newborn infants. In a few cases of diabetes the erector pili muscles show heavy deposits when the stores elsewhere in the skin are almost completely depleted. Lichner<sup>50</sup> states that in the depigmented animal all glycogen disappears except traces in stratified epithelium and in cartilage. Both these tissues are avascular.

Certain of the histological preparations in the earlier series (S. W.) were paralleled by chemical determinations carried out by Trimble and Carey.<sup>51</sup> They found the average sugar content (total reducing substances) of blood, muscle, and skin to be in nondiabetics 126, 61, and 69 mg per cent and in diabetics 238, 83, and 163 mg per cent respectively. Changes in blood sugar were much more closely followed by skin sugar than by muscle sugar.

It is perhaps premature to attempt to correlate variations in cutaneous

sugar and glycogen with the well known susceptibility of the diabetic patient to cutaneous infections. One of us (S. W.) has seen several instances of skin and muscle infection with *L. coli* in which there was sufficient production of gas from the carbohydrate present in the tissues to produce crepitation and so simulate gas bacillus infection. Two of these cases resulted fatally, and bacteriological studies both post mortem and in vitro showed only coliform bacilli and staphylococci to be present. This type of infection we have never seen in nondiabetic patients. Curiously enough the original injury and infection of the skin in both these cases resulted from scratching incident to pruritus showing this may be a dangerous as well as an annoying symptom.

**Brain.** Brain tissue does not lose the power of converting glucose to lactic acid in diabetes. But little of the lactic acid produced is formed from glycogen most being formed directly from blood sugar.<sup>11</sup> What little glycogen is present in the brain is probably immobilized as it does not follow pneratectomy.<sup>12</sup> Kerr and Ghantous<sup>13</sup> found an average of 0.1 per cent in dog brain and 0.05 per cent in rabbit brain. Munzer<sup>14</sup> demonstrated stainable glycogen in human retinal ependyma, choroid plexus neurohypophysis. In the present series glycogen has been found once in cortical cells (No. 13224) occasionally in the ependyma and choroid (14) found glycogen in the cortical cells of 14 out of 19 cases dying of the coma.

The pigmented iris epithelium of the eye may contain large quantities of glycogen associated with the vacuolated appearance of the cells with considerable fragility of the iris, a fact well known to ophthalmic surgeons (see Chapter 13).

**Pituitary.** Meinedess<sup>15</sup> noted much glycogen in the anterior pituitary particularly of 3 cases. Munzer<sup>16</sup> noted some in the neurohypophysis. Stains for glycogen were not done in the present series as the material was used for serial sections.

**Placenta.** Examination of placenta from diabetic mothers has revealed no significant difference in glycogen storage from that of normal mothers (See also Chapter 23).

**Leukocytes.** Glycogen granules were found in the leukocytes of the granulocytic series in 18 cases of diabetes, all of which showed extensive deposits elsewhere typical of diabetes. This finding is less significant as

TABLE 1. CYTODIFFERENTIAL STAINING

Type of treatment	Verm		Wright		Walcott	
	No.	%	No.	%	No.	%
Normal	1	~	1	33	1	~
Insulin	1	10	1	10	1	20
Insulin + diet + exercise	1	9	2	75	1	40
Cortisol (1 + 150 mg)	2	20	5	50	1	12

monstrable. With terminal blood sugar levels below 300 mg per cent no glycogen was found in the kidneys.

**Voluntary muscles.** Many specimens of muscle came from legs amputated for gangrene. They therefore may possibly be of dubious value owing to the influence of an impaired circulation which would certainly interfere with normal cellular function and might well influence the function of glycogen storage. However the muscle was always taken from as near the site of amputation as was possible without obtaining traumatized tissue. In this way healthy tissue could be obtained. Thanks are due to Dr I. S. McKinnick for permitting the use of fresh surgical material from his cases. In the autopsy material portions of the pectoralis major were utilized.

TABLE 18—GLYCOGEN CONTENT OF VOLUNTARY MUSCLE

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	No.	%	No.	%	No.	%	No.	%
No insulin			1	14	5	72	1	14
Insulin	1	8	3	23	9	60		
Insulin infection present	1	1	21	39	30	40	15	20
Control (non diabetics)			7	26	12	44	8	30

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Muscle glycogen has not so great significance for the diabetic as liver glycogen since only liver glycogen is directly transformable into glucose available for use of all the cells of the body.<sup>47</sup>

**Skin.** Lunn<sup>48</sup> has called attention to the presence of glycogen in normal skin. Recent work suggests that relatively little is present under usual circumstances.

When it is present the epithelium of the stratum corneum, sweat glands, sebaceous glands, and hair follicles contains most. Becker<sup>49</sup> speaks of much glycogen in the duct cells of sweat glands in newborn infants. In a few cases of diabetes the erector pili muscles show heavy deposits when the stores elsewhere in the skin are almost completely depleted. Fisher<sup>50</sup> states that in the depancreatized animal all glycogen disappears except traces in stratified epithelium and in cartilage. Both these tissues are avascular.

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diabetic coma.

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TABLE 11. GLYCOGEN CONTENT OF LEUKOCYTES

Type of treatment	None		Slight		Moderate		Marked	
	No.	%	No.	%	No.	%	No.	%
No insulin	1	~	5	33	1	20	1	40
Insulin	1	10	1	90				
Insulin effect absent	1	9	2	76	4	12	1	1
Control (diabetics)	2	20	8	80				

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Insulin	1	8	1	23	9	61		
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Type of treatment	Normal		Normal		Moderate		Marked	
	No.	%	No.	%	No.	%	No.	%
Normal	1	7	1	33	1	20	1	40
Insulin	1	10	1	90				
Insulin + diet or pancreatin	3	9	25	76	4	12	1	1
Control (no treatment)	2	90	8	80				

most of the cases were ones with evidence of sepsis. Glycogen is usually present in pus cells.

**Other tissues.** Medwedeff<sup>22</sup> and Geipel<sup>24</sup> record the occasional presence of glycogen in various tissues, e.g. thymus.

**Value in Diagnosis.**—The value of this means of histological diagnosis is well shown by a case (No. 3928) where the existence of diabetes was established by the abnormal glycogen distribution. Miss A. P., aged sixtynine years, was supposed to have had mild diabetes, but the diagnosis was never definitely established. In November 1928 she developed a left hemiplegia and gradually grew worse with mental deterioration. She showed no sugar in her urine, but this was probably due to the low caloric intake, which was little over that which would be allowed a moderately severe diabetic. Death occurred February 9, 1929, and in autopsy was performed three hours post mortem.

The chief cause of death was multiple foci of necrosis of the cerebral cortex secondary to arteriosclerosis. There was chronic pancreatitis with sclerosis and hyalinization of the islands of Langerhans.

Best's carmine stain showed a slight amount of glycogen in the heart and voluntary muscle, a trace in the liver cells, much in the liver-cell nuclei, and a moderate amount in the epithelium of Henle's loops. On these findings a diagnosis of diabetes mellitus was made.

Not only is the glycogen distribution of considerable aid in establishing a diagnosis of

example Case entered the hospital un-

a gradual downhill course of two years' duration with relatively little food intake. She had been advised to enter the hospital several times previously but refused until practically in extremis. Essentially negative except for weakness was found in her possession and she

amounts for the past two years. December 10 at 11 A.M. Cheyne-Stokes respirations were noticed, her blood pressure was too low to read, her pupils were still contracted. The nurse stated that during the past three and a half weeks the patient had not taken more than a small fraction of a maintenance diet. December 10 at 11:30 A.M. 1000 cc. of hypertonic glucose solution were given subcutaneously. Subsequently a catheter specimen of urine showed 2 per cent sugar but no acetone or diacetic acid, with blood sugar of 670 mg. per cent. No previous urine specimen had been examined owing to her incontinence of urine and feces. The finding of this urine sugar led to the suspicion of diabetes. Death occurred on December 10 at 9:10 P.M.

However, at autopsy twelve hours afterward the pancreas was found to be normal; there was abundant glycogen in the liver and in the muscles; there was no glycogen in the liver-cell nuclei or in the kidneys. In other words, the glycogen distribution was that of a normal individual, not of a

diabetic. The probability is that the absorbed glucose could not be metabolized by an individual so near death. The absence of glycogen in the epithelium of the kidney tubules in the presence of a recent moribund is probably due to the same fact. The length of time post mortem cannot explain the absence of glycogen here. There is no visualization of the renal epithelium which persists in presumptive evidence of the presence of glycogen at the time of death until disintegration of the cells. Moreover the known value of glycogen in the diabetic patient and the striking evidence of the part insulin plays in the deposition of glycogen adduced in these histological studies emphasize the importance of an adequate dosage of insulin brought out by the decrease of glycogen storage in insulin treated cases when a point appears. This of course parallels the long recognized observation that tolerance drops during infections.

**Glycogen Storage Disease**—Glycogen storage disease with hepatomegaly is one of the outstanding physical signs in most puzzling. Von Gierke<sup>10</sup> reported a case with 10.4 per cent glycogen in the liver and 6.5 per cent in the kidneys. Lush<sup>11</sup> one with 14 per cent liver glycogen. Although death usually occurs during childhood and diabetes is not present ordinarily in glycogen storage disease such as those of Parnis and Wagner<sup>12</sup> Gyar<sup>13</sup> Birn and co-workers<sup>14</sup> and Stetson and Ohler<sup>15</sup> However these cases leave something to be desired in the way of completeness. Wachstein<sup>16</sup> has reported a case of glycogen storage disease with marked hyperplasia of the islands of Langerhans and has in addition made a careful study of the distribution of acid and alkaline phosphatase which he found to be not significantly different from the normal. It is by no means clear whether this disease represents a defect in the glycogenolytic enzyme system<sup>17</sup> a pituitary hypophyseal dysfunction<sup>18</sup> or something else. Parnis<sup>19</sup> is indicated by Buser<sup>20</sup> it may be due to a deficiency of the normal catalyzed hyperphemic factor of the cells. Landing and Bingle<sup>21</sup> point out interesting similarities between glycogen storage disease and diabetes such as the familial occurrence in both the prevalence of diabetes in the relatives of some patients with glycogen storage disease and the alleged coexistence of the two diseases. However the two diseases are still insufficient to indicate any fundamental relationship between the two diseases. Landing and Bingle<sup>21</sup> point out interesting similarities between glycogen storage disease and diabetes such as the familial occurrence in both the prevalence of diabetes in the relatives of some patients with glycogen storage disease and the alleged coexistence of the two diseases. However the two diseases are still insufficient to indicate any fundamental relationship between the two diseases.

### MUCOPOLYSACCHARIDES

Recent work suggests that the mucopolysaccharides those ubiquitous substances which are components of the ground substance and framework of the liver.



membranes of connective tissue and blood vessel walls may play an important role in diseased states especially in those which appear to involve collagen primarily and in the so-called degenerative diseases. Indeed from a broadly speculative point of view practically all the histological lesions associated with diabetes may be interpreted as representing a disturbance in metabolism or storage of polysaccharides either relatively simple one (glycogen) or more complex varieties (mucin polysaccharides). (See Chapter 28 for discussion of this point.)

### SUMMARY

On the whole it may be said that there are many gaps in our knowledge of the histochemistry of carbohydrate metabolism especially as regard the localization of glycogen deposits. Indeed the deposition of glycogen in such apparently bizarre and meaningless places as the iris epithelium of the eye nuclei of liver cells and cells of the islands of Langerhans is difficult to correlate with any known facts of carbohydrate metabolism.

Letterer<sup>29</sup> has recently written an interesting review of the problem of storage and storage diseases. He proposes the following classification based on von Gierke's term thesaurismosis: (1) thesaurosis or physiological storage (2) thesauropathy or diseases of cells tissues or organs as a consequence of storage of a particular material i.e. true storage disease and (3) pathothesaurosis or pathological storage in primary metabolic diseases (this he also calls *Stapelungsdistrophie* or *deposition dystrophy*). Presumably the peculiar glycogen deposits in diabetes mellitus come under the third classification.

It is to be hoped that some of the newer methods<sup>70-74</sup> for histochemical demonstration of polysaccharides and enzymes may clarify some of the histological manifestations of aberrant carbohydrate metabolism.

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## Chapter 6

# PATHOLOGICAL EVIDENCES OF ABNORMAL FAT METABOLISM

As noted in the preceding chapter old boundary lines between the metabolic cycles of carbohydrate, fat and protein are being broken down by recent work which shows the essential interdependence of these processes. However, certain morphological accumulations of lipids may conveniently be studied under this heading.

The frequent occurrence of lipemia and of hypercholesterolemia in diabetics has long been known and very carefully studied.<sup>1,2</sup> Naturally less attention has been attracted by the rather rare abnormal deposition of lipoid material in various organs, or rather in the reticulo-endothelial cells.<sup>3</sup>

## I THE SPLEEN AND RETICULO-ENDOTHELIAL SYSTEM

This deposition when present is more marked in the spleen than in other organs. The rarity of the condition in its full blown state is striking. It was encountered in only 13 out of 527 diabetic autopsies in the earlier series.

Much of the following discussion of lipoid deposits in the spleen has been taken from an article by Root and Warren.<sup>4</sup>

While in the average spleen removed at autopsy there is little evidence either gross or microscopic of lipoid content. Pischavsky<sup>5</sup> demonstrated fat to be present in spleens removed from cases of many different diseases and to be more abundant in those spleens containing amyloid and hyaline masses. This fat is present in small droplets and gives the characteristic staining reactions for neutral fat. He further states that in children the fat is chiefly within the epithelioid cells of lymph nodules of the spleen while in adults it is found in the pulp trabeculae, capsular and vessel wall.

Kusunoki<sup>6</sup> added further proof of the frequent though inconspicuous occurrence of lipoid in the spleen and showed that most was contained in cells of the reticulum. The lipoid gave the following staining reactions: red with Sudan III, black with osmic acid and blue or blue-violet with Nile blue sulphate. Kusunoki also found the number and prominence of the lipoid-containing cells in the spleen to run parallel with the lipoid content of the blood.

Aside from this fairly common finding of rare lipoid-containing cells in the spleen, there are a few cases of diabetes with lipemia recorded in which very marked hyperplasia of these cells occurs and the lipoid present does not as a rule give the staining reactions usually encountered.

The first instance of this striking change in the spleen was mentioned by Coats.<sup>7</sup> The patient, suffering from diabetes with lipemia, had died in coma. Large lipoid-containing cells made up most of the splenic tissue. Other cases have since been reported and a general discussion is given by Schmitt.<sup>8</sup>

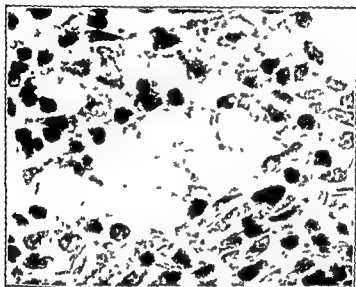


FIG. 49.—Lipoid lipomatosis of spleen. (Case M18. Male, aged sixteen years. Duration of diabetes five and a half years. Death in coma with marked lipemia.) Osmic methylene blue stain.  $\times 500$ .

Several examples of this condition have been available for study in this laboratory, including material furnished through the kindness of Drs. I. B. Mallory, I. M. Rubinstein, and R. H. Lillie.

It was noted in these cases that while the spleen was most striking, the reticulo-endothelial cells elsewhere were involved. So far as can be judged from the staining reactions, the lipoid is present in various forms, and sometimes even varies in the same case, though cholesterol esters are related substances predominate. Thus, in one case the larger droplets are presumably soaps or fatty acids, among which, according to the reaction with osmic acid, are unsaturated fatty acids. The smaller droplets contain cholesterol esters.

A case of generalized xanthomatosis was reported by Lubarsch<sup>9</sup> in a soldier aged twenty-six years with diabetes of two years' duration who developed a marked lipemia, an enlargement of the liver, and in the disease. At autopsy a small pancreas with atrophic islands was found, as well as widespread xanthomatous infiltration.

One of the puzzling features of this condition is the variation in chemical constitution of the lipoids taken up by the cells of the reticulo-endothelial system and the difference between these lipoids and those of the blood. Of course the staining reactions are not entirely reliable criteria but in several of the cases of this condition there is marked difference in the reactions of the lipid material in the circulating blood and that present in the reticulo-endothelial system. The selective absorption of cholesterol compounds and phosphatides seems not impossible.

Anitschkow<sup>10</sup> produced a somewhat similar picture in the spleen of animals by experimental feeding with cholesterol.

In Schuller-Christian's disease<sup>11</sup> cholesterol and its esters are deposited especially in the membranous bones. This has no definite association with diabetes mellitus. The lipid stored in Gaucher's disease is chiefly keratin.<sup>12-15</sup> In the lipid histiocytosis of diabetes the spleen never attains the large size of Gaucher's disease.

The essential splenohepatomegaly of Niemann-Pick must be distinguished from the lipid cell hyperplasia encountered in diabetes. In this former condition the lipid-laden cells are present in the pulp.<sup>16-18</sup> Here the lipoids are chiefly phosphatides. The material present in the diabetic lipid histiocytosis is somewhat different: cholesterol, fatty acids and neutral fats predominating with a small amount of phosphatides if we may trust the rather uncertain results of staining reactions. An excellent review of the condition in diabetes is given by Smith.<sup>16</sup>

As one of us (S. W.) has previously pointed out, atheromata of the aorta are very frequently associated with this condition in diabetes regardless of age. Thus in the case of Oppenheimer and Lilberg<sup>17</sup> atheromatous patches were found in the intima of the aorta and in the endocardium of a girl aged six years. Atheromata were found in the aorta of our Case I only sixteen years of age and our Case II thirty-three years of age died of infarct of the heart resulting from marked atheromatosis and sclerosis of the coronary arteries with involvement of the aorta as well. Case III aged twenty-one years showed numerous atheromatous patches in the intima of the aorta as did Case IV aged twelve years.

## II THE SKIN

The association of xanthoma of the skin and diabetes has long been known, xanthoma diabeticorum being a familiar phrase to every medical student.

It is important to distinguish between xanthelasma (xanthoma palpebrarum) and xanthoma diabeticorum.<sup>18</sup> Xanthelasma refers to benign slowly growing flat yellowish tumors of the medial portion of the eyelid usually bilateral and not occurring much more frequently in diabetics than in the general population. Microscopically they are made up of fat-con-

aining macrophages but may not show as many Touton giant cells\* as other types of xanthoma. Xanthoma diabeticorum which is called xanthoma eruptivum by some authors<sup>20, 21</sup> and not sharply distinguished from xanthoma tuberosum by others<sup>22</sup> is apparently definitely related to hyperlipemia. Thannhauser<sup>22</sup> classifies it as secondary eruptive xanthoma associated with symptomatic hyperlipemia. It occurs as a tumor like lesion often brilliant yellow associated with a red periphery which is characteristic. The lesions may appear quite suddenly and are usually not numerous on the outside and back of the forearm and especially about the elbows and knees where they may be confluent. Histologically they are indistinguishable from xanthoma tuberosum being made up of lipid containing macrophages with occasional Touton giant cells. They may however show more peripheral inflammatory reaction and more fibroblastic proliferation than xanthoma tuberosum and if biopsy is taken when the lesion is undergoing involution cholesterol and other lipoids may be found extracellularly as well as intracellularly. (Figure 50.)

In this diabetic series true xanthomas are extremely rare in spite of the fact that hypercholesterolemia is fairly frequent.<sup>23</sup> There appears to be considerable variation in the cholesterol fat ratio in these lesions.

However occasional cases a section from one of which is shown in Figure 50 show greater relative amounts of cholesterol. In this case cholesterol made up 45 per cent of the lipid present though only 16 per cent of the total mass. Curiously enough the tumors in this patient disappeared as rapidly under dietary and insulin treatment as do the ordinary xanthomas.

Examination of the eyelids by Waite and Beetham<sup>24</sup> showed 59 xanthelasma among 4001 diabetic eyes (1.5 per cent) and 7 among 914 nondiabetic eyes (0.8 per cent).

In 4 of Joslin's<sup>16</sup> 12 cases of xanthoma diabeticorum lipemia retinalis was also present. Arcus shows no definite association with hypercholesterolemia.

The highest blood cholesterol value associated with xanthoma found in this laboratory is 781 mg. per 100 cc. In practically all cases of xanthoma reduction of the fat in the diet has reduced the blood lipid and has resulted in the disappearance of the xanthoma. One of Wile's<sup>25</sup> cases was a diabetic. When the patient was placed on a practically fat free low-calorie diet the multiple xanthomas which he had decreased markedly in size. Later the patient was placed on a relatively high fat diet on which he became sugar free and the tumors showed an even more rapid disappearance. On resumption of normal diet the xanthomas reappeared.

The presence of true xanthoma is a strong hint that the diet needs revision from the standpoint of lowering the lipid intake. Adlersberg *et al*<sup>27</sup> believe that the tendency to xanthoma is inherited as an incomplete

\* This is a giant cell in which the nuclei lie near the center of the cell grouped around a small island of nonfoamy cytoplasm and surrounded by foamy cytoplasm.<sup>18</sup>

dominant trait in persons who carry two abnormal genes for cholesterol and that atherosclerosis is also frequent in these people.

Still another factor to be considered is that the diet of the average diabetic patient is rich in lipochromes and local cutaneous lipid deposits may very well be emphasized by deposition in them of lipochromes. Connor<sup>11</sup> found the normal range of carotinemia to be from 0.0 to 0.07 mg. and he found in 18 of Joslin's diabetic patients selected at random values ranging from 0.05 to 0.16 mg. Miss Hunt in this laboratory has found in 19 determinations on diabetic patients who were clinically suspected of carotinemia values ranging from 0.09 to 0.86 mg. Whether carotin has anything to do with the peculiar yellowish color of the bones in some diabetics<sup>12</sup> is not clear.

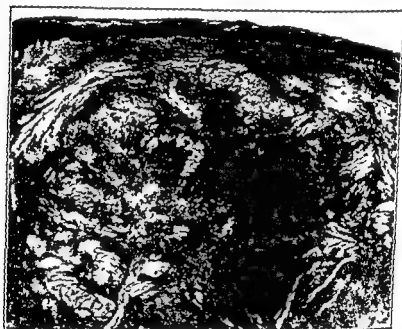


Fig. 50. Surface of wall of skin of virgin rabbit composed of cholesterol crystal and fibrous connective tissue. Male age forty-eight years. Duration of diabetes eight years. Lesions similar to that above cleared up on insulin treatment. H&E stain.  $\times 20$ .

Necrobiosis lipoidica diabetorum was first described by Oppenheim<sup>13</sup> but named by Urbach.<sup>14</sup> Twenty-five cases, most of them young, occurred among Joslin's<sup>15</sup> patients.

The disease is not confined to diabetics, although about 90 per cent of the reported cases have been diabetics, mostly women and usually poorly controlled. According to Hildebrand *et al.*<sup>16</sup> the gross lesions may go through four phases: (1) a small reddish infiltrated papule; (2) an increasingly

large lesion becoming violaceous (3) a slightly raised firm yellow plaque which may soften and sometimes ulcerate and (4) a flattened yellowish lesion with central atrophy and depression and a peripheral irregular ring red or violaceous region of infiltration. Microscopically there is robustness of collagen with homogenization and degeneration of fibroblasts and Fisher<sup>22</sup> stress the pillar of the necrobiotic areas in the hematoxylin-stain stain, also loss of elastic tissue and a variable peripheral cellular reaction sometimes having a tuberculous appearance. The lipids which were not present in the necrobiotic areas in all their cases stained brownish red with Sudan III and were only rarely doubly refractile. The lipids are characteristically extracellular in this condition. Calcification of the subcutaneous tissues<sup>23</sup> appeared in the legs of two elderly diabetic women. (Alumina was present both in the fat and in the corium). Their blood chemical findings other than blood sugar were essentially normal. Diabetes had been present for fourteen and six years respectively. No explanation has been found.

### III THE GALL BLADDER

Inasmuch as the bile is the chief route for elimination of cholesterol from the body and many gall stones are of the cholesterol type abnormalities of the gall bladder might be expected as a result of the disturbed lipid metabolism in diabetes.\* Table 20 shows the incidence of gall stones and cholecystitis in a group of 453 autopsied diabetics over thirty years of age and in 500 nondiabetic autopsies in the same age group.

TABLE 20—FREQUENCY OF GALL BLADDER LESIONS IN AUTOPSED CASES OVER THIRTY YEARS OF AGE

Number of cases of lesion	Diabetic		Non-diabetic	
	453	%	500	%
Cholecystitis without stone	133	29	100	20
Cholecystitis with stone	28	6	107	21
No lesion	1	0.2	2	0.4
	1	0.2	0	0

TABLE 21—RELATIVE INCIDENCE OF GALL STONES AT DIFFERENT AGES (Diabetics)

Age yrs	Number of cases	Percentage of total
20 to 30	3	1.2
30 to 40	11	4.2
40 to 50	33	9.9
50 to 60	53	11.9
60 to 70	62	13.7
70+	11	19.1

\* It is recognized that disturbed lipid metabolism may not be the only cause of the apparent increased incidence of gall bladder disease in diabetes. The exact pathogenesis of diabetes may also be an important factor. However it is convenient to discuss the gall bladder in this chapter.



Table 21 from Ophuls<sup>25</sup> shows the incidence of gall stones by age groups in 3000 autopsies, of which only 34 were performed upon diabetics.

The available statistics as to the occurrence of gall stones vary greatly from 3 per cent in White's<sup>26</sup> series of 11 031 autopsies based on Guy's Hospital material to 21 per cent in Mentzer's<sup>27</sup> series of 600 autopsies at the Mayo Clinic. Kaufmann<sup>28</sup> at Basle found 10.9 per cent of 11 021 autopsies to show gall stones.

Wilder<sup>29</sup> reported 16 cases of cholelithiasis and 4 of severe cholecystitis in 58 autopsies on diabetic patients performed at the Mayo Clinic or 28 per cent with cholelithiasis and 34.5 per cent with gall bladder disease.



FIG. 51.—Gall stones largely cholesterol from Case DA2795 Female aged sixty years. Duration of diabetes five years. These stones were found at autopsy. There had been no definite symptomatology pointing toward them.

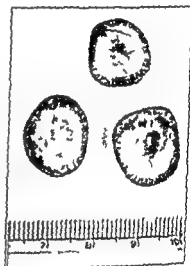


FIG. 52.—Cross sections of several of the stones shown in Figure 51. Slightly enlarged.

This compares closely with our findings (31 per cent of 453 autopsies showing cholelithiasis and 36 per cent showing either cholelithiasis or cholecystitis).

This increased prevalence of cholelithiasis and cholecystitis in diabetes is of obvious importance from two angles—the gall bladder disease in itself and the marked depression of sugar tolerance exerted by any inflammatory process in the diabetic organism.

In view of the stress laid on gall bladder disease as a possible cause of pancreatic injury, and hence diabetes it is of interest to consider whether diabetes or gall bladder disease appeared first in the cases. In most instances the onset of diabetes antedated the appearance of symptoms referable to the gall bladder disease. This of course is of little value as evidence as the stones were silent in many cases. Moreover, Joslin's cases with more detailed clinical data showed the gall bladder disease to develop first. Relatively few of the cases examined post mortem showed sufficient pancreatic change of inflammatory type to admit pancreatitis due to gall bladder disease as a cause of the diabetes, although practically all show some pancreatic fibrosis of slight to moderate degree.

In a few instances, as Case A51-17 (Peter Bent Brigham Hospital) the gall stones in all probability predisposed to repeated attacks of acute pancreatitis of mild type with eventually sufficient injury to the islands of Langerhans to permit diabetes to develop. This case occurred in a male aged fifty-seven years. For five years attacks of pain in the right upper quadrant recurred at intervals with occasional periods of jaundice. One month before death from bronchopneumonia diabetes of moderate severity developed. At autopsy three large stones were found in a thick-walled gall bladder. The pancreas showed patches of fat necrosis and fibrosis. The islands were hypertrophied.

However, cases of this type are rare. In Table 22 the occurrence of gall stones in the earlier series is compared in various periods of duration of the diabetic process.

TABLE 22 — RELATION OF GALL STONES TO DURATION OF DIABETES

Duration of diabetes years	Per cent with gall stones
0 to 5	
6 to 10	20.4
11 to 15	36.6
15+	41.3
?	33.3
	21.2

Of course the known mildness of gall stone diabetes may account for the strikingly increased occurrence of gall stones in the cases of longer duration.

The association of cholelithiasis with arteriosclerosis is also marked. Of course this might well be explained by the tendency of both to occur in the upper age groups, but the strikingly high incidence of both these lesions in diabetes suggests a common origin, possibly in the disordered fat metabolism.

Joslin<sup>10</sup> cites a striking case No. 310 who developed diabetes at the age of seventeen years. When the patient was only twenty-one years of age gallstones were definitely diagnosed. Death occurred in coma at thirty-eight years of age.

The bulk of stones found are of the cholesterol type, though occasionally the pigment-calcium stones are encountered. A few examples of analyses from a series of diabetic and nondiabetic gall stones performed by Dr. J. Du Preux in this laboratory are given below and illustrate the marked

TABLE 23—ANALYSES OF GALL STONES (DRY WEIGHT)

Case No.	Diabetes	Number of stones	Cholesterol per cent	Calcium per cent
793	—	1	96.4	
4319	+		0.15	34.6
Average	+		93.2	1.5
Range	+		91.5	0.02

variation in chemical constitution that may occur among stones from diabetics. However, the frequent occurrence of cholesterol rich stones in the diabetic series and the statistical evidence suggest a connection between diabetes, hypercholesterolemia, and gall stones.

Wilder<sup>22</sup> felt that the unusually high incidence of gall stones in diabetes previously reported by Root and Warren<sup>14</sup> could not be substantiated by the results in his series, although his figures were comparable to theirs, as he



FIG. 53—Large single cholesterol stone with small amount of calcium at one end found in nondiabetic patient. Female aged fifty-one years.

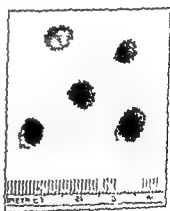


FIG. 54—Calcium and pigment stones from female, aged sixty years. Duration of diabetes 10.7 years. This is one of the few cases of diabetes showing the calcium or pigment stone rather than the cholesterol type of stone.

considered the difference in prevalence between his diabetic and control series slight. However, it must be remembered that the control series which he used for comparison was based on material from the Mayo Clinic which would naturally be weighted with cases of gall-stone disease.

There is no one type of stone that occurs to the exclusion of others as the ones well show although if any could be picked out as typical one may consider it the large or old stone made up of a mass of radiating cholesterol crystals with or without a small amount of calcium and pigment. Fatty livers in diabetics are known to have a disturbed fat metabolism and stones are more common in diabetic than in nondiabetic individuals.

## IV THE LIVER

One of the oldest references to the pathology of diabetes is to the marked fatty infiltration of the liver that it is sometimes present. \* Medicine called attention to this in 1746. Adelsberg and Porges<sup>12</sup> consider an enlarged and fatty liver as a regular finding in diabetes. Fenger<sup>13</sup> states that the fat content of the normal liver is 1 to 2 per cent of its total weight but that under pathological conditions the fat may increase to 40 per cent or more.

TABLE 24 WEIGHTS OF DIABETIC LIVERS

Part A

Liver Weights—Cases up to January 1, 1910

Age yrs	Total	Blood	Normal	Abnormal
0 to 9	5	1	4	0
10 to 19	9	3	6	0
20 to 49	18	9	9	22
50+	111	28	45	38
	143	41	62	60

Part B

Liver Weights—Cases January 1, 1910 to September 1, 1911

Age yrs	Total	Blood	Normal	Abnormal
0 to 9	0	0	0	0
10 to 19	6	0	0	0
20 to 49	13	3	10	1
50+	14	4	11	1
	215	50	81	81

Part C

Liver Weights—Cases September 1, 1911 to September 1, 1915

Age yrs	Total	Blood	Normal	Abnormal
0 to 9	2	normal	0	2
10 to 19	6	0	0	6
20 to 49	45	6	12	27
50+	271	6	113	91
	324	12	125	126

\* Here again it is recognized that a fatty liver is not necessarily a sign of disturbed fat metabolism—it may well be the injury of the blood to some other cause.

In Table 24 the livers are grouped by weight and age. For weights under twelve years of age those of Coppoletta and Wolbach<sup>46</sup> are considered normal for ages 13 to 19 a range of 900 to 1400 grams for adults the range from 1400 to 1800 grams has been accepted as normal. The table is divided into three parts: the first made up of those cases dying prior to January 1, 1930, the pre-insulin cases and those of the early Banting Era, and the second and third parts of those cases dying since that date. Most of the latter received more liberal carbohydrate diets and more insulin. However the liver weights are strikingly similar in the three series. While there is a slight trend toward heavier livers in the diabetic patients, this is far from striking.

While it has been generally assumed that the increase in size and weight of the liver in diabetes is due to fat, this does not exclude other factors. Enlargement of the liver has been shown to be due to glycogen storage to circulatory changes, amyloidosis, and metastatic tumor as well as fat. Of much importance is the fluid content of the liver. Best<sup>47</sup> has noted that the liver water content tends to diminish as the fat content increases.

Chinin<sup>2</sup> emphasizes the frequent occurrence of fat in the Kupffer cells in the liver and holds that the amount may be related to the severity of the diabetes.

The more recent series of autopsies contains a high proportion of coronary deaths; hence passive congestion of the liver may be a factor.

The abdominal pain not infrequently associated with a large liver in the diabetic has been suggested by Marble<sup>47</sup> as secondary to stretching of the capsule. This suggests a rapid change in size—perhaps too rapid for fat deposition—but quite compatible with marked increase in intracellular fluid.

In a series of diabetic and nondiabetic livers analyzed in this laboratory by Dr. Nellie Halliday, the total fatty acid ranged from 2.2 to 4.3 per cent wet weight of fresh liver in the nondiabetic cases and from 4.1 to 10.8

in the diabetic cases.

formalin fixed liver

The possibility of fatty degeneration of the liver in toxemias due to infections accompanying the diabetic state must be considered, but this might explain only a limited number of the cases.

As has been already mentioned, large amounts of fat in the liver do not preclude the presence of normal or even large amounts of glycogen. Fatty infiltration may occur in von Gierke's disease.<sup>48</sup>

The fatty livers found in experimental diabetes<sup>49</sup> are of much interest. Dragstedt's assumed insular hormone, lipocaine, is effective in preventing fatty infiltration in the livers of depancreatized dogs when given in

Possibly the supply of this hypothetic hormone might be credited to the  $\alpha$  or D cells the functions of which thus far are unknown. However in a few cases whose liver weights were above normal no demonstrable change was found in any of the insular cells.

Considerable question has been raised in the recent literature as to the existence of lipocur and its possible identity with choline or other fatty acids.<sup>32-34</sup>

The effects obtained by feeding raw pancreas lecithin<sup>35</sup> or choline<sup>36</sup> or betaine hydrochloric acid<sup>37</sup> permit survival of depancreatized insulin-treated animals without appreciable hepatic abnormality.

Large livers were noted by clinical examination in 12 of 44 juvenile diabetic patients and in only one of 233 diabetics over twenty years of age (Hanssen<sup>38</sup>).

(Clinical evidence of the effect of lipocur has been presented by Gravel and Radwin<sup>39</sup> who found that regression of enlarged livers in three diabetic children was obtained with lipocur and on discontinuance of its use enlargement reoccurred in one to two months. They also noted that the decrease in size of the liver was paralleled by a lower total serum lipid.

Marble<sup>40</sup> et al. concluded choline is not a major factor in controlling hepatomegaly as the diets of the children with hepatomegaly they followed continued adequate choline and good response was obtained from the better regulation of their diabetes through protamine zinc insulin.

It is interesting that the hepatomegaly of galactose diabetes seems to be due to fat.<sup>41</sup>

A useful discussion of the various diseases including those involved in the liver from the clinical and physiological standpoint has been presented by Thurnham<sup>42</sup> and by McLenen and Patterman.<sup>43</sup>

Probably the most extensive work to date on the liver in that of Zimmerman et al.<sup>44</sup> They biopsied the liver in 28 diabetics with uncomplicated well-controlled diabetes. Sixteen of these showed vacuolization of liver cell nuclei presumably representing glycogen. Fourteen patients showed fatty metamorphosis in varying degree and in 3 of these cirrhosis was found. In 8 of the patients with fatty change there were significant amounts of iron-containing pigment in the liver cells. No relation could be established between the liver changes and previous control of the diabetes.

In a further study<sup>45</sup> the same authors applied the glucose-insulin tolerance test of Himsworth to the same patients. They found that a positive correlation existed between the occurrence of fatty metamorphosis and insensitivity to insulin also a positive but not conclusive correlation between fatty changes on the one hand and age obesity and duration of diabetes on the other. They were unable to attribute the fatty change to nutritional deficiencies, alcoholism or inadequacy of control of the diabetes. They speculate that pituitary hyperactivity might be responsible for both the fatty metamorphosis and the insensitivity to insulin.



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## Chapter 7

### THE PATHOLOGY OF ACIDOSIS AND COMA

Acidosis and coma in diabetes are associated with the accumulation of the so-called ketone bodies which are no longer regarded as abnormal metabolites but are known to be formed normally and abundantly in the course of fatty acid degradation. They do not accumulate in the blood of normal persons because they are normally consumed in muscle and liver as rapidly as they are formed. When glucose is scarce either because of lack of dietary carbohydrate or because of loss of glucose in the urine or when glucose though abundantly present is not being catabolized at a normal rate excessive quantities of fat are transported from the depots to the liver and there degraded probably to two-carbon acetyl fragments and a portion of these are converted to acetoacetic acid. If these normal processes become sufficiently exaggerated the rate of acetoacetic acid formation will exceed the body's capacity to destroy this compound and its level in the blood will rise. The re-establishment of carbohydrate metabolism in such a subject appears to relieve the liver of the necessity of degrading fatty acids at excessive rates and as the formation of ketone bodies resumes its normal rate the ketone bodies which had accumulated in the body are either excreted in the urine or destroyed by the tissues of the body in their normal fashion (Stetten).

The pathological physiology of diabetic coma appears to be exceedingly complex especially as regards electrolyte metabolism and it is only in recent years that an insight has been gained into such factors as the all important role of potassium. Butler\* in particular has pointed out that many deaths have occurred after correction of the acidosis and after adjustment of the serum concentrations of sodium chloride and glucose to normal with no demonstrable cause of death being apparent at autopsy. As he indicates such deaths may well have occurred in the past as a result of therapy being focused on extracellular fluid concentrations at the expense of intracellular needs particularly the need for potassium.

The rapidity of the development of acidosis and its sequel coma depends not only on the rate and amount of production of acid bodies but also on the amount excreted and the rate of excretion which are closely linked with the condition of the kidneys as well as the amount of acid bodies taken up by the tissues of the blood with displacement of the combined carbon dioxide.

As diabetics have a striking tendency to develop arteriosclerosis renal vascular disease intercapillary glomeruloclerosis and pyelonephritis the kidneys are frequently already impaired when needed to deal with the main

tenance of acid base equilibrium. Thus acidosis may be somewhat more likely to develop than in normal individuals even given an equal production of acetone bodies.

The culmination of acidosis is coma. The pathological changes in coma are conspicuous by their absence. Even in pre-insulin days when diabetic coma developed in uncomplicated cases of diabetes there were no characteristic pathological changes that would enable one at post mortem or on looking at a section of tissue to say. This material came from a case of diabetic coma. Today the same holds true.

Now the changes produced by acidosis of the acetone body type are so far as can be seen but little different from those seen in the acidosis so readily produced by intoxication of animals with various mineral acids by the prolonged administration of hydrochloric acid. Tubular damage due to toxic injury of the epithelial cells is a very frequent finding and still more impairs renal function thus furthering the development of a vicious circle. This is the cause of the showers of casts in the urine so frequently associated with acidosis.

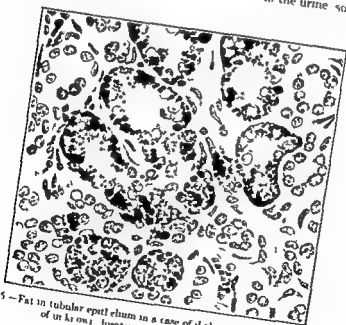


FIG. 55—Fat in tubular epithelium in a case of diabetes with hyperkalemia. Diabetes of unknown duration. Sudan II stain.  $\times 500$ .

In cases of coma one sees at their best the various forms of pathological distribution of glycogen together with the absence of normal deposits which were discussed in Chapter 5. In fact some authors have endeavored to explain the development of coma on the basis of glycolytic infiltration and vacuolization of the cortical cells. Although we have not been able to

perform satisfactory glycogen stains on cerebral tissue from many cases of diabetic coma in the few cases that we have examined we cannot substantiate this theory.

The antagonism between septic processes and insulin autogenous or injected is all too well known clinically. A diabetic patient treated or untreated loses tolerance with even minor infections. This is as true today as in the pre-insulin days. When attacked by infection an insulin treated diabetic requires more insulin during the course of the intercurrent disease and promptly drops back to his former requirements when the infection is past. The tables in Chapter 5 bring out vividly the fact that the one anatomically visible feature of carbohydrate metabolism glycogen storage tends to approach that of the normal individual in insulin treated cases but that in cases with sepsis the picture tends toward that of the untreated diabetic in spite of insulin. Whether this is a direct antagonism it is impossible to say.

Rare cases have been reported in which the liver showed normal or increased glycogen content after death from coma.<sup>4</sup> Usually hepatic glycogen is depleted. Popper and Wozisek<sup>5</sup> give liver glycogen values ranging from 75 mg. to 19 mg. in 8 cases of coma with most values toward the lower extreme. As noted in Chapter 5 Bondy and Sheldon<sup>6</sup> found only a trace of glycogen in a liver biopsy from a case of coma but noted restoration of glycogen within a few hours of treatment.

Let us consider the typical pathological findings in a case of acidosis progressing to death from coma in a non insulin treated diabetic. The only changes which we find are those characteristic of prolonged acidosis—practically nothing but renal tubular changes sometimes with evidence of toxic injury to the liver cells.

The kidneys are responsible for severe functional disturbances and some of the cases at autopsy show varying degrees of acute tubular damage often with marked fatty degeneration<sup>7</sup> of the tubular epithelium. Anuria or oliguria is not infrequent.<sup>8,9</sup>

Rarely renal insufficiency may be so marked as to prevent the excretion of the acetone bodies in coma so that ketonuria may not appear.<sup>10</sup> Nitrogen retention may be very marked and showers of casts and albumin may be present in the urine.

However the changes in the epithelium of the renal tubules in cases dying of coma are not comparable with the acute damage of toxic origin. The necrosis is less marked the absence of polymorphonuclear leukocytic exudate is striking.

The liver cells are practically devoid of glycogen their nuclei distended with it. The epithelium of the kidney tubules shows large amounts that in the heart muscle is increased while in the striated muscle and skin it is depleted.

The pancreas may show almost any type of lesion in the islands or in the acinar tissue or it may show none. Fulminating cases of diabetes rapidly

progressing to coma and death frequently show hydropic change of the islands.

One fairly frequent post mortem finding is the occurrence of blood fresh or partly digested in the stomach and intestinal tract. Usually no bleeding point can be found and presumably capillary hemorrhages rather than gross bleeding points are the source. At times the stomach in particular is tremendously dilated causing marked distress. This distention may be a

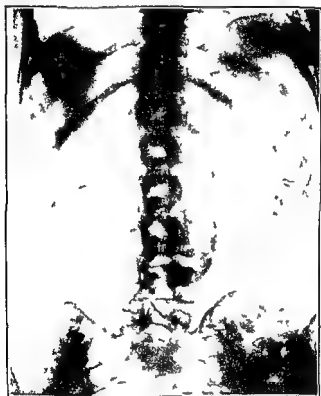


FIG. 56.—Extreme acute dilatation of the stomach in diabetic coma. Photograph by courtesy of Prof. Dr. H. Berning.

of acute gastric dilatation in diabetic coma has been emphasized by Berning, one of whose illustrations is reproduced in Figure 56.

Lesions similar to those of acute asphyxia—capillary dilatation, pericapillary edema, degeneration of ganglion cells—have been reported by

Dillon Riggs and Dyer<sup>2</sup> in the brains from 8 fatal cases of coma. This capillary dilatation may help to explain the frequent mucosal gastrointestinal

the changes in cases of coma. Charni illustrates not too convincingly similar changes in a single case. We have not found these changes. Windle *et al*<sup>15</sup> found no definite cytologic changes in guinea pigs made severely acidotic by ammonium chloride given by stomach tube by lactate buffer given intravenously or by breathing 30 per cent carbon dioxide.

The eye changes in coma are discussed in Chapter 16.

A few of these coma cases were accompanied as would be expected by disturbed metabolism of the higher lipid substances. Of the 11 cases of abnormal lipid distribution mentioned in Chapter 6, 5 died in coma.

Lipemia in coma may be most striking; the blood may resemble melted strawberry ice cream with a total lipid of 19.9 per cent. When studied by dark field microscope such a blood shows myriads of droplets. Zon and Warren<sup>16</sup> studied the chilo-micron count in a number of normal and diabetic patients but found no relationship to cholesterolemia. However the highest counts occurred in diabetics. (See also Chapter 9.)

The doughy consistency of the abdomen frequently noted in coma is due usually to two factors: distention of the colon by retained feces and distention of the stomach. Often dilatation of the bladder may be extreme.

Striking evidence that the anatomical changes in uncomplicated coma are reversible and hence short of actual cell destruction is given by the clinical course. Given a case of coma pure and simple, insulin treatment brings prompt recovery, far more rapid than would have been possible if any marked injury were present.

The development of insulin treatment has strikingly altered the picture in so far as deaths from coma are concerned.<sup>17</sup> This is well shown in the table in Appendix B.

Acute or subacute pancreatitis was associated with several of our cases. Four of these were under twenty years of age. Loord and Bowen<sup>18</sup> have reported 2 cases of acute pancreatitis in young diabetics dying with coma.

Little attention seems to have been given by clinicians to acute pancreatitis as a possible precipitating cause of coma in an established diabetic. Its occurrence is emphasized by Root.<sup>19</sup>

The skin of coma patients may be very susceptible to infection. Riven<sup>20</sup> has described dermatitis gangrenosa occurring in coma.

Coma with or without infection may be accompanied by a high white cell count which greatly complicates the picture from the clinical standpoint. A white cell count as high as 97,000 with frequent myelocytes has been reported<sup>21</sup> in coma without any evidence of infection and the blood picture returned to normal and remained so after recovery from coma.

Recent studies on electrolyte changes in diabetic acidosis will doubtless

prove of great importance in the understanding of the condition although as yet they are without known pathologic counterpart. Holler was apparently the first to recognize the importance of potassium deficiency and his paper has been followed by many others confirming his observations.<sup>22, 24</sup> Martin and Wertman<sup>23</sup> studying 14 cases of severe diabetic acidosis found a marked fall in serum potassium in 46 per cent and a considerable drop in serum magnesium in 36 per cent. The prime importance of the electrocardiogram in assessing the disturbance of potassium metabolism is indicated in Chapter II. Franks *et al.*<sup>25</sup> found that although the plasma inorganic phosphorus was elevated in all their cases prior to treatment a precipitous fall occurred in both the plasma level and urinary excretion of phosphorus when treatment was begun suggesting a depletion of stores of this element. Butler<sup>2</sup> has recently written an able summary of the pathologic physiology and management of diabetic coma emphasizing particularly the importance of considering the intracellular as well as the extracellular electrolyte balance.

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## Chapter 8

### DIABETES AND INFECTION

INFECTION was the cause of death in 213 of the 818 fatal cases of diabetes. It is a striking fact that the incidence of death from infection in the cases summarized in the last edition of this book was 38 per cent, while in the cases added since that time (1938) the incidence is only 11 per cent, a change doubtless attributable largely to the modern antibiotics. Tuberculosis and syphilis are omitted from the total number only the acute infections being considered. Eighteen additional deaths were due to tuberculosis and 2 to syphilis. Moreover a number of cases of gangrene are included only those cases being considered where generalized sepsis or gas gangrene was the cause of death. Pneumonia was responsible for more deaths than any other localized infectious condition. There were 38 deaths from bronchial pneumonia and 10 deaths from lobar pneumonia.

Just as arteriosclerosis is the bugbear of the diabetic who is doing well so infection is the fear of the diabetic who is doing poorly. The more severe the diabetes the more susceptible he is to infection. Once a diabetic is infected his diabetes becomes far more severe. As stated previously sepsis is the greatest foe of insulin.

Whether or not we can translate to man the result of the experiment of Rosenthal and Behrendt<sup>1</sup> who found that fresh pus mixed with insulin abolished the insulin effect when it was injected into rabbits nevertheless clinical observation has long shown that there is a marked increase in the severity of the disease during infection. As Joslin<sup>2</sup> states: "Insulin will not maintain a sugar free urine during an active infection unless given in very large doses." Moreover when these large doses are given there is the constant fear that with sudden recovery in insulin reaction may result.

Inasmuch as many cases of the acute infectious diseases show central or focal necrosis of the liver or an acute renal tubular necrosis it is reasonable to suppose that other parenchymatous organs such as the pancreas are acted on as well particularly where the pancreas of the diabetic is just on the ragged edge its islands barely producing enough insulin to keep the organism going and needing to be helped out with either dietary control or extraneous insulin. It is obvious that any very slight infectious process or toxemia may be sufficient to injure the island cells. Thus the already too delicate balance that is being maintained is overthrown.

We have seen this toxic injury to the islands in a number of instances both in nondiabetic and diabetic patients. One of the most striking cases is that of a nondiabetic patient who died from lobar pneumonia. One of

us (S. W.)<sup>1</sup> had the opportunity of studying this case No. 10 while in Dr. Mallory's laboratory. Most of the cells in the islets of Langerhans were necrotic or showed some evidence of injury. There were as well numerous mitotic figures sometimes as high as seven in a section of a single island showing that there had been serious damage. It is quite possible that cases such as these may give the clue to the transient glycosurias that occasionally appear in nondiabetic patients in the course of severe acute infections.

As previously suggested it is perfectly reasonable to explain the severe drop in sugar tolerance noted in diabetic patients during acute infection in this same way. The one hopeful feature is that the diabetes is not made permanently worse or rather the sugar tolerance is not kept at the same low level to which it is forced during the course of the infection. The quick recovery of the diabetic patient after the infection has gone is very striking indeed suggesting that if the effect is on the insular epithelium it stops short of actual cell necrosis.

It should be added that the evidence is by no means clear that the above mechanism is operative nor if it does indeed occur that it is the only one involved. Other possibilities are listed by Marble<sup>4</sup> as follows (see also Greene and Keohene<sup>5</sup>): (1) increased production of hormonal antagonists; (2) destruction of insulin by enzymes in leukocytes or pus; (3) interference with the storage of glycogen. None of these mechanisms can be regarded as being supported by anything like adequate evidence.

Also Menkin<sup>6,7</sup> studying the mechanism of enhanced diabetes associated with inflammation in depancreatized dogs concluded that there was increased proteolysis at the site of inflammation and that the products of this increased protein catabolism seemed to lead to increased local glucose-neogenesis from protein. He also stated that insulin appeared to repress both the increased proteolysis and the gluconeogenesis. This work requires confirmation.

The types of infection vary but pneumonia and pyogenic infections stand out above all others.

To protect the diabetic against pneumonia there is relatively little that we can do except to maintain his diabetes in as favorable a condition as possible. If anything it is necessary to be overcareful with regard to colds and upper respiratory tract infections in the hope of checking them before pneumonia may have an opportunity to develop.

After pneumonia the pyogenic infections are the most important particularly those of the staphylococcus and the streptococcus groups. An intact skin is essential to a diabetic patient for it is his best safeguard against infection. Blisters and scratches that would be insignificant to the normal person must be carefully guarded from the access of bacteria. Even organisms which ordinarily are of very low virulence are capable of setting up severe infections. Thus *Staphylococcus albus* is ordinarily considered of little significance and indeed if found in a blood culture is generally



dismissed as a contaminant yet it may be an extremely dangerous organism to the diabetic responsible for pyemia and multiple abscesses just as in certain cases in children it may be responsible for osteomyelitis.

The diabetic skin is fertile soil for the pyogenic bacteria. The carbuncle with its tendency to develop septicemia with the subsequent establishment of multiple abscesses is one of the most dangerous types of cutaneous infection. Since these organisms are always present in the skin waiting for an

course of tremendous importance in both reducing the number of organisms present on the skin and in keeping in check their opportunities for entering wounds.

Pyogenic infections caused 102 deaths. In few cases aside from the fulminating osteomyelitis of children is there as widespread distribution of abscesses as that found in some of these cases. Fortunately thanks to the modern antibiotics such cases are becoming rare.

Except for their extent and tendency to become generalized furuncles and carbuncles in diabetic patients differ in no regard from those in non-diabetics.

Infection and gangrene are a particularly dangerous combination to the diabetic. Infection is not only a frequent complication of gangrene due to the low resistance of the undernourished and necrotic tissue leading frequently to the establishment of generalized sepsis but it may be the underlying cause as well. Even a low grade infection may be sufficient to overcome the narrow factor of safety barely maintained in the diabetic leg with arteriosclerosis of the diabetic type and result in the establishment of the gangrenous process.

An odd finding secondary to high sugar content of the blood and tissues is encountered in a diabetic with pulmonary infection due to *Aspergillus*. Large amounts of oxalates were excreted in the urine and numerous crystals of calcium oxalate were present in the sputum.<sup>9</sup>

Three cases of mucormycosis of the central nervous system were reported by Gregory *et al*<sup>10</sup> in diabetics. These cases were remarkable in that all represented the same triad: (1) uncontrolled diabetes, (2) evidence of orbital infection and (3) meningoencephalitis associated with the presence of large nonseptate hyphae. An entirely similar case involving hemorrhagic infarction in addition was described by LeCompte and Meissner.<sup>10</sup>

Excess sugar in the urine may lead to pneumaturia when a sugar fermenting organism gains access to the bladder.<sup>11</sup> Air introduced by catheter must be ruled out.

Two very striking and unusual cases called attention to the clinical importance of the high sugar content of the blood and tissues in cases of diabetes with infection. Both these cases had been clinically diagnosed as gangrene but repeated cultures both aerobic and anaerobic as well as

guinea-pig inoculation failed to reveal anything other than colon bacilli and staphylococci. In an additional case formation of gas by organisms in a perinephric abscess outlined the kidney strikingly in the roentgenogram.



FIG. 27. Roentgenogram of infected knee of diabetic patient showing gas outline in soft tissues around the joint.

Case No. 3747 was a man aged fifty-eight years whose diabetes was known to have existed for only a month, although undoubtedly it had been present for some time previously. He had suffered from intense pruritus and through scratching had produced several infected wounds in the inguinal regions. The cause of death was an extensive phlegmonous abscess involving the inguinal region and the left flank. At post-mortem, as in examination during life, there was marked crepitation of the tissues, but a considerable amount of rather creamy pus together with a definitely purulent condition of the underlying muscles, coupled with repeated cultures negative for *Clstridium welchii* and positive for *Escherichia coli*, led us to

## DIABETES AND INFECTION

believe that the latter was the offending agent. Cultures and animal inoculations at post mortem also failed to produce any organism other than the colon bacillus. This strain of *Escherichia coli* was an extremely active gas producer. Unquestionably this organism in the sugar laden tissues had succeeded in producing the amount of gas that had proved so confusing from the clinical standpoint.

Focal infections are of considerable importance in keeping down sugar tolerance and are as frequent in diabetic as in nondiabetic patients. Teeth, tonsils and gall bladder are all possible points of localized chronic inflammatory processes. Here again the pathology is similar to that of non-diabetics.

Gingivitis is as frequent an accompaniment of diabetes as is caries. Kent<sup>12</sup> felt that periodontoclasis (pyorrhea alveolaris) was more common in the diabetic than the nondiabetic person. When diabetic children are brought under control progressive dental caries is arrested.<sup>13</sup>

The importance of infections of the urinary tract has been emphasized by Sharkey and Root<sup>14</sup> who found 18 per cent of 196 diabetic autopsies to show purulent infection of various portions of the tract and by Baldwin and Root<sup>15</sup>. The frequency of chronic and acute pyelonephritis is emphasized in Chapter 12. Prostatic abscess when clinically obvious is highly dangerous to the diabetic patient.

The reasons for the increased susceptibility of diabetics to infection are far from clear. Marble *et al*<sup>16</sup> discussed the following as possible causes: (1) increased sugar content of blood and tissues (2) decreased activity of blood elements associated with resistance to infection such as (a) subnormal activity of complement (b) subnormal phagocytizing capacity of leukocytes (c) subnormal bacteriostatic and bactericidal action of whole blood (d) inadequate functioning of fixed tissue cells (4) lowered capacity of tissues to react to antigenic stimuli (e) lowered state of general cellular nutrition.

Present-day opinion is opposed to the simple explanation that the increased sugar in blood and tissues promotes more rapid growth of organisms since there is no valid evidence to support this point of view. As regards the other possibilities the evidence is also inconclusive. Marble *et al*<sup>16</sup> found that fresh defibrinated blood and heparinized whole blood of diabetics had essentially the same phagocytic bacteriostatic and bactericidal power against selected strains of streptococci as did blood from normal controls. The results in individual cases could not be correlated with the duration, severity or state of control of the diabetes.

Richardson<sup>17</sup> concluded from testing the ability of diabetic and non-diabetic blood to kill *E. typhosa* that complement in diabetics does not differ from that in nondiabetics but that the blood of diabetics had a slightly weaker bactericidal property and that the diabetic patients were less able than the nondiabetics to form agglutinins. However the differences were not striking. Later<sup>18</sup> he found that underfed rabbits with a

depleted liver glycogen developed lower agglutinative titers against typhoid vaccine than did well fed controls with abundant liver glycogen. In further experiments the same author<sup>8</sup> found an apparently significant correlation in normal rabbits and depancreatized cats between the percentile amount of glycogen in the liver and the survival time after intravenous inoculation of bacteria. Likewise bacteria were found more frequently in the organs of depancreatized cats twenty four hours after intradermal inoculation of bacteria. However alterations in blood sugar, cholesterol, proteins, and liver glycogen did not appear to influence this dissemination.

Recently Cruickshank and Payne<sup>9</sup> found that a Type II pneumococcus inoculated into whole blood of rabbits with alloxan diabetes and incubated *in vitro* grew much more rapidly than in normal blood. However when plasma alone was inoculated growth tended to be heavier in the normal plasma. They therefore suggest that there may be a defect in the leukocytic defense of animals with alloxan diabetes.

Flick *et al.*<sup>11</sup> testing the antibody response of diabetic patients to typhoid immunization found a positive correlation between the antibody response and the serum albumin concentration.

## SYPHILIS

While syphilis, particularly in the congenital form with its tendency to involvement of the liver and pancreas, is a possible etiological factor in the production of diabetes, nevertheless the incidence of demonstrable luetic pathology is very low. We have not seen a pancreas in this series where it seemed that syphilis could be responsible for the changes present, and even the occurrence of syphilitic lesions in other organs is very rare. Warthin and Wilson<sup>12</sup> emphasized the importance of syphilitic pancreatitis, but our material is apparently very different from theirs.

In our series 2 deaths only were due to the disease. One case, a colored female aged fifty years with diabetes of three years' duration, died of hemorrhage from a syphilitic ulcer of the trachea. The other case, a male aged forty-two years, died from a ruptured abdominal aneurysm. His diabetes had been present for three months.

## TUBERCULOSIS

Although tuberculosis was formerly responsible for many of the deaths of diabetic patients, only 18 deaths from tuberculosis occurred in this series. This is probably abnormally low, as cases with a long-continued pulmonary tuberculosis are often discharged to their homes or to sanatoria.

This is in marked contrast to a generation ago when nearly half the fatal cases of diabetes died from tuberculosis. However the decrease in rate of incidence of tuberculosis in adult diabetics is not as rapid as the decrease of tuberculosis in the general population.<sup>13</sup>

The course of tuberculosis complicated by diabetes seems to be in no way remarkable as long as the diabetes is properly controlled<sup>24-26</sup>. A curious observation in regard to the pathology is that of Wiener and Kaye<sup>27</sup> apparently confirmed by Root and Bloor<sup>28</sup> that dense pleural adhesions seem to be only about half as frequent in diabetics as in nondiabetics. Apparently this is correlated with clinical recognition of greater ease of inducing pneumothorax in diabetics with tuberculosis. However, since diabetes is not known to influence granulation tissue or fibrosis one wonders whether this finding may not indicate a selection of less slowly progressing cases (one thinks of pleural adhesions as associated with tuberculosis of fairly long duration).

For extended information on tuberculosis in diabetes reference may be made to the chapter by Root in Joslin's book.<sup>29</sup>

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## Chapter 9

### VASCULAR DISEASE IN DIABETES

DURING the last few years a shift in emphasis has occurred with regard to diseases of the blood vessels in diabetes. While sclerosis of the larger arteries, especially the leg vessels and the coronary arteries, is still recognized as a major problem, careful observation of diabetics who have survived ten, fifteen, twenty, or more years with their disease makes it increasingly clear that the disabilities in this group are largely referable to disorders of the smaller blood vessels (*i.e.* arterioles, venules, capillaries).<sup>1-4</sup> The lesions affecting these small vessels seem to be fundamentally different from those involving the larger elastic and muscular arteries, and in many respects they seem to be characteristic of, if indeed not specific for, diabetes.\* Since, however, the effects of involvement of the small vessels are usually manifest in either the kidney or the eye, discussion of this subject will be found in the chapters devoted to the *eye* organs and to the *eyes* of long duration (Chapters 12, 13, 19). The present chapter will be concerned with disease of the larger arteries, *i.e.* atherosclerosis and medial calcification (Monckeberg's sclerosis).

#### ARTERIOSCLEROSIS OF THE LARGER VESSELS

Whether the increased prevalence of arteriosclerosis in diabetes is due to the disease itself or to methods of treatment of the disease, the importance of the problem is such that it cannot be disregarded. In the first edition of this book the statement was made: "I have yet to see at autopsy a diabetic or to read the autopsy protocol of a diabetic who's disease has lasted five years or more, free from arteriosclerosis, regardless of age." Since then a few cases with duration over five years have been found to be practically free from arteriosclerosis, but they are not many.

Of course, many of our cases fall in the older age group and therefore would be expected to have arteriosclerosis in any event. However, the cases in the younger age groups frequently show this lesion, as has been pointed out in Chapter 6 in considering the pathological evidences of abnormal fat metabolism. If we accept tentatively Aschoff's<sup>5</sup> modification of Virchow's imbibition theory of arteriosclerosis, which in essence seems to

\* It is unfortunate that the word *arteriosclerosis* is used so loosely to include the retinal and renal lesions of diabetes, especially in the young patient. The characteristic mucronecrosis and irregular glomerular changes seem to be in fact diseases of glomeruli.

## VASCULAR DISEASE IN DIABETES

be still the most popular theory of pathogenesis of the disease it is obvious that blood plasma laden with lipid will predispose to the development of arteriosclerosis and the lesion may be correlated with abnormal metabolism.

In the days when excessively large amounts of fat were given to diabetic patients a direct cause and effect relationship between the ingested lipoids and the fatty plaques of the aorta and other large arteries seemed so clear that little question was raised concerning it. Today, however, after almost thirty years of insulin and relatively low fat diets we are still faced with a disturbingly high incidence of coronary occlusion in diabetics and atherosclerosis accounts for a much higher percentage of deaths among diabetics than among nondiabetics.

In Chapter 19 it is shown that fully 43 per cent of a series of 107 patients who had had their disease for over 15 years died as a direct result of atherosclerosis of the coronary arteries. Most of the rest of course had atherosclerosis although it was not a direct cause of death. This figure is to be contrasted with a study of a large general autopsy series (predominantly nondiabetic) in which only 4 per cent of deaths were directly attributable to disease of the coronary arteries (see Chapter 11). Also as shown strikingly in Appendix B deaths attributable to arteriosclerosis rose from a mere 17.5 per cent in the early years of diabetes to the staggering figure of 69.4 per cent in recent years (1944 to 1949). In other words atherosclerosis emerges without question as the leading cause of death in diabetes accounting for over two thirds of the fatalities.

Atherosclerosis is such an important cause of death in nondiabetics also that a tremendous amount of effort is being devoted especially in the more recent literature to attempts to elucidate the mechanism by which the characteristic intimal plaques are produced. The history of the disease has passed through many phases and as will be noted below the present phase is dominated by the study of cholesterol and the lipoproteins. It is because of the general importance of the disease and because there seems to be no difference in kind in the atherosclerosis of diabetics and nondiabetics that much of the discussion in this chapter contains no direct reference to diabetes.

Atherosclerosis is most easily studied at the autopsy table but it is obviously of great importance to develop clinical methods for detecting its presence, activity, progression or regression in the patient before death.

In the examination of the living patient there are numerous ways in which the presence of arteriosclerosis may be detected. The method of palpation of the arteries is old one is almost tempted to say as old as it is uncertain. The examination of ocular fundi gives an excellent index of arterial disease in many cases but no one has yet shown how close the correlation between retinal and systemic vascular disease may be.

Morrison and Bogun<sup>8</sup> have shown the great help that the roentgen ray may give in the diagnosis of arteriosclerosis with calcification.

However, this diagnostic method can take no cognizance of atheromatous or other changes even though they may be very extensive, since it records the deposition of calcium salts.\*



FIG. 58. Roentgenogram of leg of 21-year-old diabetic male showing calcified artery.

By these methods White<sup>9</sup> found evidence of arteriosclerosis in 24 of 104 diabetic children. 19 showed calcification of leg arteries and 5 showed retinal sclerosis alone. Since her earlier report she has used the roentgen ray effectively in gauging the presence of vascular disease not only in children but also in pregnant diabetic women (Figs. 58 and 59).

\* Calcification of the vessel lumen is also common in diabetes.<sup>10</sup> Its relation, if any, to arterial disease is obscure.



Recently Landbom<sup>10</sup> has published a beautiful demonstration of the value of refined arteriographic techniques in the study of vascular disease in the legs. An extensive earlier study was that of Ruhl.<sup>1</sup>

Our other means of diagnosis are clinical—the physical signs and symptoms of local circulatory deficiencies. Some of these are of definite value such as the pain in claudication, the occurrence of gangrene, color changes, the presence or absence of pulsation in the vessels, and local temperature. Goodman *et al.*<sup>11</sup> in a critical survey of methods of assaying vascular disease in diabetes found the following techniques of greatest value: (1) carotid sinus pressure test,\* (2) palpation of supraclavicular pulsations



FIG. 59—Calcified pelvic arteries in a 21-year-old diabetic woman.

(3) presence of calcification of the abdominal aorta and in the arch of the aorta, (4) determination of occlusive changes in the legs by palpation.

It must be emphasized that all methods for the diagnosis of generalized arteriosclerosis in the living patient are subject to gross error.

There are two main types of arteries—the elastic arteries such as the aorta, the iliac and carotid arteries, and the muscular arteries such as the posterior tibial or brachial, in which the development of the muscular is very often overshadowed by that of the elastic lumens. This muscular type is encountered chiefly in the peripheral and the splanchnic arteries. The arterioles also are of the muscular type.

\* The conclusion of this author is that the alleged value of the carotid sinus test in detecting arteriosclerosis is greatly exaggerated. In particular, the test is of little value in the detection of peripheral disease.



is some doubt about this mechanism. These calcium salts become the calcareous or even bony plaques formed in the atheromatous regions. As the plaque becomes larger and reaches nearer the surface the blood stream may sweep away portions of the pulturneous material forming the familiar atheromatous ulcers.

As will be noted further below Lansing Blumenthal and co-workers have maintained in a series of papers<sup>15-18</sup> that atherosclerosis is actually closely associated with the process of aging and that the formation of atheromatous plaques is always preceded by calcification (elasto-calcinosis) of the media.

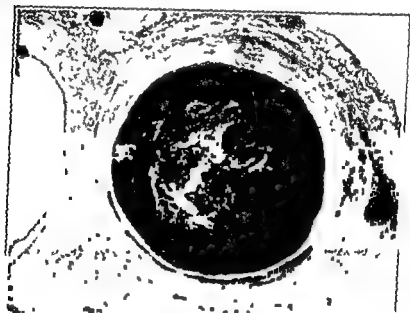


FIG. 61.—Femoral artery showing medial calcification or the Mönckeberg type of sclerosis. The dark mass filling lumen of the artery is a thrombus.

The second great type of arterial change is that seen in the muscular vessel. Here medial calcification, the Mönckeberg type of sclerosis predominates. This lesion is entirely distinct from the atheromatous process which we meet in the elastic vessels and is restricted to the muscular vessels. Intimal change is negligible and the lumen remains intact or is even actually widened. The calcium becomes deposited in fine granules in the connective tissue stroma about the muscle cells. This calcification gradually becomes more and more abundant finally surrounding and causing atrophy of the smooth muscle cells. It is sometimes difficult to tell whether the particles of calcium salts are outside or within the muscle cells which may be necrotic. Any leukocytic reaction is extremely rare. Not

infrequently organization of the calcified mass may take place with resultant formation of bone which may even contain marrow in the more advanced cases. Through secondary intimal injury thrombosis may occur as shown in Figure 61. This is the type which is demonstrated in the roentgenogram.

There seems to be little, if any, relationship between the occurrence of this type of arteriosclerosis and the atheromatous although occasionally the two forms may coexist. (As noted above, one group of investigators holds that there is a close and important relation between calcification in the media and atheromatous plaques.) In the smaller arteries there may be

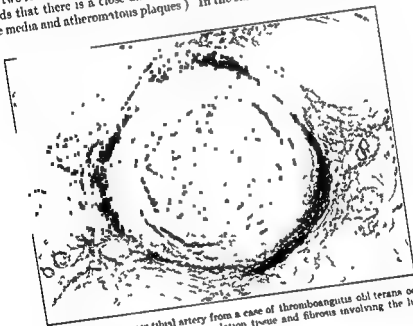


FIG. 62. The anterior tibial artery from a case of thromboangitis obliterans occurring in a nondiabetic. Note the granulation tissue and fibrous involving the lumen with some recanalization.

thickening of the intima with or without the deposit of lipoids. When this intimal change is at all marked one is justified in considering the possibility that the patient from whom the vessel was removed may be diabetic.

The third great type of arterial change is that known as thromboangitis obliterans or Buerger's disease. This type usually occurs in young adults. Here thrombotic organization of the clot, and subsequent partial recanalization of the thrombosed vessels are the outstanding features followed by shrinkage of the walls and resultant reduplication of the elastic laminae. Calcification is absent as is deposition of lipoids. There is usually a very sharp distinction between the fairly normal-appearing adventitia and musculature and the obliterated fibrotic lumen. An excellent example is shown

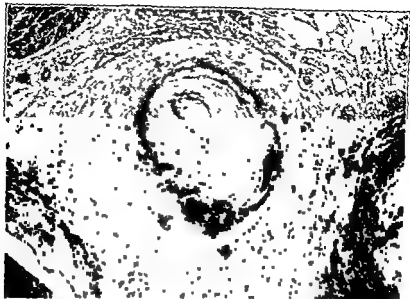


FIG. 63 — Arteriosclerosis involving the anterior tibial artery in a diabetic. Note the marked intimal thickening. There is also a considerable degree of medial calcification. Hematoxylin-eosin stain.  $\times 100$



FIG. 64 — Roentgenogram of the left leg of the diabetic boy whose legs were illustrated in the previous picture. In the present picture the boy is twenty-one years of age. There is marked calcification of the vessels. Note the failure of the leg to develop normally in addition to the dwarfism.

in Figure 62. It is apparently not more frequent in the diabetic than in the nondiabetic.

In the diabetic individuals the atheromatous type of lesion is the outstanding one. The others may be present but if there is one thing that should lead us to suspect that an artery in a given case comes from a diabetic patient it is when we find the intimal type of involvement in the muscular arteries either alone or superimposed upon the medial calcification.

In a series of 816 diabetic autopsies suitable for study of vascular lesions 288 deaths (35 per cent) were due to arteriosclerosis or tissue changes immediately secondary to that lesion. Since 124 cases of the series were



Fig. 62.—Masses of cholesterol crystals from thickened intima of anterior tibial artery. Male aged sixty-four years. Duration of diabetes unknown. Intimal change is accompanied by a moderate amount of medial calcification. Eosinophilic stain.  $\times 375$ .

under forty years of age, this high prevalence of arteriosclerosis as a cause of death is even more striking. In the 300 cases of the first series 72 (24 per cent) died from arteriosclerotic lesions. This slight relative increase is due probably to the sharp drop in deaths from coronary disease. The percentage in cases of long duration is practically unchanged. Of the 157 cases of diabetes of fifteen years duration or over 94 or 60 per cent died from arteriosclerotic lesions.

Of interest is Case No. 1296, a female, aged fifty years, who showed almost no arteriosclerosis although her diabetes had been present for fifteen years.

The heart led all the other organs with 201 of the arteriosclerotic deaths attributable to it. Of course, this is only a small proportion of the hearts showing the evidences of arteriosclerotic injury, as reference to Chapter 11 will show.

TABLE 25 — INCIDENCE OF ARTERIOSCLEROSIS BY DECADES

<i>Age at death yrs</i>	<i>Total cases</i>	<i>Total with arterio-sclerosis</i>	<i>Deaths due to arterio-sclerosis</i>	<i>Arterio-sclerosis absent</i>
0 to 10	11			11
11 to 20	35	17		18
21 to 30	40	30	4	10
31 to 40	38	34	5	4
41 to 50	88	82	14	6
51 to 60	197	195	65	2
61 to 70	257	255	129	■
71 to 80	132	132	66	
81 +	14	14	■	
?	4	3		1
	816	762	288	54

TABLE 26 ARTERIO-SCLEROTIC DEATHS AMONG 816 DIABETIC AUTOPSIES

<i>Arteriosclerosis most marked in</i>	<i>Number</i>	<i>Per cent</i>
Heart	201	61.8
Extremities	2	9.4
Brain	28	9.7
Kidneys	17	5.9
Not localized	15	5.2
	288	100.0

TABLE 27 ARTERIO-SCLEROTIC DEATHS AMONG 157 DIABETIC AUTOPSIES DURATION OF DIABETES FIFTEEN YEARS OR OVER

<i>Arteriosclerosis most marked in</i>	<i>Number</i>	<i>Per cent</i>
Heart	74	72.7
Extremities	3	3.2
Brain	10	10.6
Kidneys	6	6.4
Generalized	1	1.1
	94	100.0

Arteriosclerosis of the extremities as exemplified by fatal gangrene accounted for 27 deaths. The percentage of deaths from gangrene has been reduced to one-fourth of that in the first series. This reflects the value of educating the diabetic patient in the care of his feet and the influence of the antibiotics.

Cerebral manifestations of arteriosclerosis were responsible for only 28 deaths, and vascular disease of the kidneys although present in many cases, was fatal in only 17\*.

This marked prevalence of cardiac and peripheral arterial involvement in diabetic patients contrasts strongly with the frequency of cerebral and renal damage in nondiabetics. That this is no peculiarity of the present series is shown by Table 28 giving the site of fatal arteriosclerosis in 4,495 cases.

TABLE 28. LOCALIZATION OF TERMINAL LESION IN 4,495 DIABETIC DEATHS DUE TO ARTERIO-SCLEROSIS (JONLIS<sup>29</sup>)

Site of terminal lesion	Va n p era	Per cent of all deaths				
		Allen era	Early Banting era	Late Banting era	Hagerlorn era	Charles H. Best era
Brain	18	22	20	18	22	22
Heart	18	15	1	9	6	"
Heart	15	40	41	57	60	64
Peripheral gangrene	21	17	11	13	8	4
Unassigned site	5	4	5	4	4	3
Number of deaths	5 <sup>2</sup>	203	172	1474	1654	131

Cerebral arteriosclerosis is no more frequently a cause of death in diabetic patients than in nondiabetic in spite of the greater prevalence of arterial disease in the former group.

Taking the aorta as an index of arteriosclerosis we find only 66 free from arteriosclerosis among the 783 which are recorded. Of these 155 showed slight involvement, 191 moderate and 365 marked involvement. This tabulation includes cases of all ages though naturally most lie in the older age groups.

### ARTERIO-LAR SCLEROSIS

Disease of the arterioles usually takes the form of hyaline thickening of the intima, i. e. the change so often associated with hypertension in the nondiabetic and described in the classical paper of Moritz and Oldt.<sup>30</sup> Sclerosis of the arterioles in the diabetic causes its most obvious damage in the kidneys. Discussion of this type of vascular change is therefore given in greater detail in Chapter 12.

### DISEASE OF THE CAPILLARIES AND VENULES

As indicated at the beginning of this chapter, disease of the smallest blood vessels has now come to occupy the center of the stage in discussion of the vascular complications of diabetes. For consideration of these lesions the reader is referred to Chapters 12, 13, and 19.

\* In the group of young diabetics of long duration renal disease is a major cause of death (see Chapters 12 and 19).



## ETIOLOGY OF ATHEROSCLEROSIS

No part of the whole field of pathology has been so much the domain of dogmatic statement as arteriosclerosis. Each generation in its turn has accepted some doctrine put forth with authority on the most insecure basis in fact and even now, as in some religious discussions, there are those who are insistent upon some explanation quite at variance with that equally vigorously maintained by another group.

The above statement by a distinguished student of the disease<sup>1</sup> appeared in 1933 and is still applicable today. Only the flimsiest sort of evidence is available as to the causes of arteriosclerosis and one can do little more than list various hypotheses.

In this section discussion will be limited to the intimal type of disease—atherosclerosis.\* As indicated above this is the most important type of vascular disease since it now appears to be the leading cause of death not only in diabetes, but in the rest of the population as well. No consideration will be given to theories unsupported by any valid scientific evidence such as those which hold the disease to be due to aging alone, tobacco, alcohol, syphilis, hypotension, lead, epinephrine, etc. Some idea of the tremendous amount of literature both good and bad which has appeared on the subject may be gained from the voluminous review by Hueper.<sup>2</sup> Other valuable recent reviews are those by Duff and McMillin,<sup>3</sup> by Katz and Dauber,<sup>4</sup> and by Gubner and Ingerleider,<sup>5</sup> to which the reader is referred for a more detailed discussion with numerous references.

The most obvious and characteristic feature of atherosclerosis is the presence of lipids in both early and late lesions. Most theories regarding the etiology and pathogenesis of the disease have therefore included some attempt to explain how the lipids get into the lesions and whether their presence is indicative of a primary metabolic disturbance or simply represents a secondary deposit in injured tissue.

One reason for the large number of theories concerning the pathogenesis of atherosclerosis is to be found in disagreement as to what constitutes the earliest lesion of the disease. Indeed it is impossible to be certain of this point since in examining the tissues at necropsy one is attempting to reconstruct a dynamic process from morphologic evidence. It is usually necessary to assume therefore that the smallest lesions are the earliest and it follows that such lesions will be found only by chance during the examination of many microscopic sections. Hence it is held variously that the earliest change is a swelling or necrosis of the intercellular ground substance,<sup>6,7</sup> a fine deposit of extracellular lipid droplets,<sup>8</sup> or a small collection of lipid phagocytes.<sup>9,10</sup>

The oldest and perhaps most widely held theory of the pathogenesis of atherosclerosis is the imbibition or infiltration theory. First enunciated by

\* The medial calcification of Mönckeberg as seen in the diabetic does not differ from the same type of lesion in the nondiabetic. The pathogenesis is quite obscure.

Virchow, and popularized by Aschoff,<sup>7</sup> the theory holds that local loosening of the intercellular cement substance due to mechanical stress is followed by imbibition of lipid-containing fluid from the blood plasma into the vessel wall. Phagocytosis of the lipids by histiocytes occurs, followed gradually by fibrosis and growth in size to form the fully developed plaque. Aschoff distinguished an atheromatosis of youth in which the lipid infiltration was reversible, but more recent authors seem to agree that this process does not differ in kind from that seen in adults.

A variant of this concept is the anoxemia theory of Hueper<sup>8</sup> based on experiments in animals with various macromolecular substances and holding that due to an imbalance of plasmatic colloidal equilibrium a thin film of cholesterol is deposited on the endothelium leading to interference with the nutrition of endothelial cells which become more permeable and are at the same time stimulated to take up cholesterol and become foam cells. Foam cells are also thought to be formed from subendothelial phagocytes.

In criticism of Hueper's theory it may be said that it is based on experimental work in which massive doses of macromolecular colloids (polyvinyl alcohol, methyl cellulose, pectin and xerica) were injected intravenously into animals. As noted by Leary,<sup>14</sup> these substances are essentially foreign bodies which in large doses overwhelm the experimental animal and lead to its death; also the intravenous route is not a natural one. Further the carry-over to the human situation has the defect of being a reasoning by analogy with insufficient supporting evidence.

The major role given to cholesterol in this theory is in accord with the modern trend which reflects the influence of the rabbit and its response to

in the human disease is indicated by the work of Hirsch and Weinhouse<sup>15</sup> who found the lipids of atheromatous plaques to be present in the same proportion as in the plasma suggesting an infiltration from the blood rather than a selective absorption of cholesterol. Burk and Rosseter<sup>16</sup> however found differences in concentration of the various lipids which suggested to them that simple imbibition was not an adequate explanation.

held that diabetes is without influence on atherosclerosis of the aorta and that the determining factors are age and blood pressure. However only 4 of their 32 diabetics could be classified as non-tensive, and one wonders about the significance of this small number.

Another point of view was expressed by Winternitz *et al.*<sup>17</sup> according to which most of the changes in atherosclerosis are regarded as mediated by the vasomotor which can be demonstrated in abundance in animal experi-

atheromatous plaques. According to this theory, the lipids and other constituents of the plaque may be derived from exudation and hemorrhage from the *vasa vasorum*. Although such processes certainly are factors in the life history of the older plaques, and in the formation of thrombi, they can hardly be regarded as effective in initiating the earliest lesions, since vessels are rare in the intima of the normal human artery. Also, it has been pointed out that the veins of humans, which are highly vascularized, and the arteries of some animals showing extensive vascularity are only rarely the seat of atherosclerosis.<sup>21</sup>

Another theory of the origin of the lipids of the atheromatous plaque is that of Leary,<sup>22</sup> who holds that invasion of the arterial wall by foam cells is the origin of the plaque. This point of view is based on years of experience in feeding cholesterol to rabbits. The sequence of events in the rabbit, according to Leary, is as follows: when cholesterol is fed, there occurs a latent period during which cholesterol esters are precipitated in crystalline form in the liver and are then taken up by the Kupffer cells, which eventually escape from the sinusoids, traverse the lungs, and selectively invade (by "chemotaxis") the intima of arteries from the flowing blood. The lesions so produced are held to be entirely similar to the lesions of human coronary atherosclerosis, although this point has been contested.<sup>23</sup> Leary also contends that the earliest stages of the lesion, *i. e.*, invasion of the endo-

the lipophages, by reason of their low specific gravity, would be expected to move toward the slowly-moving peripheral part of the blood stream, adjacent to the endothelium. This type of reasoning is, of course, highly speculative.

Leary has much evidence to support his conception of the sequence of events in the rabbit. However, the theory as applied to the human is seriously weakened by failure to demonstrate the part of the cycle involving invasion of the intima by foam cells. Leary holds that this difficulty is due to the fact that the cells, when they invade the intima, are in fixed positions, and thus are not subject to the same mitotic activity as the endothelial cells. This theory is also as weak as the one which holds that the earliest lesion in both rabbit and man may be considered debatable. In short, although Leary's hypothesis has much to recommend it, the evidence for it can hardly be regarded as complete.

**The Role of Cholesterol**—It would be difficult to overestimate the importance of the rabbit in the history of thinking on the subject of atherosclerosis. The regularity with which this animal develops vascular lesions when fed cholesterol as shown by Anitschikow<sup>28</sup> and the Russian school and later by Leary<sup>29</sup> has doubtless been responsible more than any other factor for the current emphasis on cholesterol. In general other animals have been quite resistant to cholesterol induced atherosclerosis but recently the chicken has been found to be a susceptible animal<sup>30, 31</sup> and also the dog (provided that thourieid be given at the same time)<sup>32</sup>.

This extensive experimental work in animals has led in the last few years to an extraordinary and probably unwarranted emphasis on the role of cholesterol as distinct from other lipids in human atherosclerosis. As will be seen below most papers have reported only total serum cholesterol without reference to other lipids and often without reference to genetic and other factors which may be of major importance. The recent admirable papers of Ancel Keys<sup>33, 34</sup> indicate the necessity of controlling some of the many variable factors before any valid conclusions can be drawn.

In general it may be said that efforts have been devoted toward answering two questions: (a) Is the level of serum cholesterol related to the degree of atherosclerosis in a given individual? and (b) Is the level of serum cholesterol related to the dietary intake of this substance?

As regards the first question both negative and positive evidence is available. Lande and Sperry<sup>35</sup> studied the serum cholesterol in relation to the degree of atherosclerosis (measured by lipid content) in the aortas of 123 healthy persons who died by violence; they found no correlation. Ungerleider and Gubner<sup>36</sup> found no significant difference as regards cardiovascular disease between two large groups of insurance applicants, one having blood cholesterol less than 175 mg. per cent, the other over 200 mg. per cent. In another report,<sup>37</sup> however, they found distinctly less vascular disease in a group with unusually low blood cholesterol and raised the question as to whether the so-called normal range of blood cholesterol may not be in reality high. Several other recent reports have suggested a positive correlation between the height of the serum cholesterol and the presence of coronary sclerosis. For instance, Steiner and Domaniski<sup>38</sup> followed 15 patients with coronary disease and 15 controls of comparable ages for periods up to two years and found an average blood cholesterol of 355 mg. per cent with a range of 308 to 490 in the coronary group and an average of 285 with a range of 214 to 334 in the controls. Of considerable interest is the work of Bock *et al.*<sup>39</sup> and Allersberg *et al.*<sup>40</sup> who have demonstrated a striking familial incidence of high serum cholesterol, xanthoma, xanthelasma, arcus of the cornea and coronary disease in certain families. They even suggest that xanthoma and often coronary disease develops in patients who carry two abnormal genes for cholesterol, i.e. homozygotes, whereas in those with only one abnormal gene (heterozygotes) the only

manifestation of disturbed lipid metabolism may be an elevated serum cholesterol. Wilkinson<sup>48, 49</sup> has made similar observations.

Although there does appear to be some evidence suggesting a relation between hypercholesterolemia and atherosclerosis the relation between serum cholesterol and the dietary intake of this substance is by no means clear. Heyman and Rick<sup>5</sup> found no rise in serum cholesterol after giving large doses to children. Stainer<sup>51</sup> likewise obtained negative results. Shaffer<sup>52</sup> found no increase in coronary disease in a group of patients on a high cholesterol diet. Keys<sup>10, 53</sup> whose papers stand as models of careful investigation in this field found no correlation between dietary intake and serum cholesterol (except with extraordinary diets such as rice and fruit).<sup>54</sup> Keys also points out in a convincing way some of the many factors which must be taken into account before one can say that a given individual does or does not have a normal serum cholesterol. Apparently a highly refined fat free diet (protein hydrolysate and dextrimaltose) will reduce serum cholesterol,<sup>5</sup> but the practical significance of such observations is open to question. Geographic evidence is unconvincing paradoxically both the Eskimos and the Chinese are cited as having a low incidence of arteriosclerosis the former being on a meat diet which must be fairly high in cholesterol the latter on a vegetable diet presumably low in cholesterol. Gertler *et al*<sup>55</sup> point out some of the fallacies of this type of evidence. Thannhauser<sup>56</sup> also emphasizes the weaknesses in the argument and holds that the vascular xanthoma of familial hypercholesterolemia is a different disease from arteriosclerosis. In general it may be said that in man evidence for a relation between the amount of cholesterol ingested and either the height of the blood cholesterol or the incidence of atherosclerosis is not convincing.<sup>40, 41, 48, 49, 55, 56</sup> This seems to be true even though a fairly impressive case may be made out by citing only the positive evidence and neglecting the negative.<sup>57</sup> At any rate attempts to prescribe low cholesterol diets<sup>58</sup> to prevent arteriosclerosis would appear to be premature. Best<sup>59</sup> dismisses the use of choline in arteriosclerosis as having no validity.

In this connection the work of Wilens<sup>60</sup> is of interest. He points out that overweight is common in all the important conditions that are associated with atherosclerosis and demonstrates on autopsy material a general correlation between obesity and the incidence of atheromatosis. However Faber and Lund<sup>61</sup> appear to find no definite effect of obesity when hypertension is taken into account.

One gets the impression that too much attention has been given to cholesterol alone without considering its relation to other substances particularly lipids in its absorption and metabolism especially as regards its stability in the blood stream. As noted above it has been shown that the lipids of the atheromatous lesion are present in the same proportions as in the blood plasma. They do not consist solely of cholesterol. The papers of Gubner and Ungerkader<sup>62</sup> and of Gertler *et al*<sup>55</sup> and of Duff and McMillan<sup>63</sup> are especially valuable in pointing out the multiplicity of factors

involved.\* The importance of fat in the intestinal absorption of cholesterol and the rôle played by phospholipids and proteins in maintaining the colloidal stability of cholesterol in the circulating blood are of great importance. It has been suggested that the ratio of phospholipid to cholesterol may be more important than the total cholesterol.<sup>47, 48</sup> Kellner *et al*<sup>49</sup> showed that in rabbits injection of detergents resulted in a higher blood cholesterol but less arteriosclerosis. They found that the phospholipids were elevated along with the cholesterol. Simms *et al*<sup>45, 46</sup> have described the presence of substances in plasma called lipofagins which promote the formation of intracellular fat granules in tissue culture and also produce fat deposition in segments of adult chicken arteries *in vitro*. The action of these substances is said to be opposed by antilipofagin.

Also of much interest is the recently reported work of Becker *et al*<sup>47</sup> who have studied the chylomicron count in the blood after giving a fat meal (0.5 gm. oleomargarine per kg. of body weight). They found that the chylomicron count of young subjects reached a peak at two and one half to three hours and returned to the fasting level by the end of the fifth hour. In older subjects the count did not reach its peak for eight to twelve hours and did not return to the fasting level for about twenty-four hours. These results suggest that older persons have a continuous elevation of the chylomicron count and the authors suggest a possible relation to the occurrence of atherosclerosis.

Zon and Warren<sup>48</sup> found higher chylomicron counts in diabetics than in normals but could establish no relation to the cholesterol level of the blood. Morison<sup>49</sup> has also made extensive studies of chylomicrons.

The new work of Gofman and associates<sup>50</sup> has attracted a good deal of attention. They characterized the larger molecules of the serum by ultracentrifugation, recording their results in terms of the flotation rate in Svedberg units (S). First studying rabbits they found that normally the serum contained a good many lipoprotein molecules of S<sub>2</sub> 5 to 8, with a cholesterol content of about 30 per cent by weight. In cholesterol feeding this lipoprotein increased but there also appeared giant molecules of S<sub>2</sub> 10 to 30. A study of 104 patients with proved myocardial infarction revealed an almost universal occurrence of cholesterol bearing molecules of S<sub>2</sub> 10 to 20 at fairly high levels. All their normal controls showed a lower frequency of occurrence of measurable concentrations of this class of molecule than did the myocardial infarction group. They also found significantly higher numbers of these molecules in males from twenty to forty years of age than in females of the same age group. In the age groups over forty both males and females showed significant increases in these molecules. Their data all seemed to indicate a probably higher incidence of measurable concentrations of molecules of the S<sub>2</sub> 10 to 20 class in a group of diabetics.

\* A useful recent review of the relative importance of lipoproteins in the pathogenesis of atherosclerosis is to be found in a series of articles in the *American Journal of Medicine* 1956.

On the other hand Keys<sup>76</sup> holds that there is no evidence that the estimation of these giant molecules has any special virtue beyond that of simple cholesterol measurements in predicting atherosclerosis or in estimating the activity of the arteriosclerotic process.

Finally it should be re-emphasized that arterial disease should not be considered in terms of lipoids alone to the exclusion of other well recognized factors such as mechanical stress, infection, vascularity of the vessel wall, physico-chemical characteristics of the ground substance (see Chapter 28) etc. Saphir and Gore<sup>77</sup> have recently stressed the possible etiologic role of infection in the coronary disease of young people. The work of Meyer<sup>78</sup> and of Faber<sup>79</sup> suggests that the connective tissue changes in atherosclerosis may be independent of the lipid deposits. Blumenthal Lansing *et al*<sup>80</sup> hold that calcification of elastic tissue (elasto-calcinosis) is a normal accompaniment of the aging process and is also a prerequisite for the formation of intimal plaques. This would not of course explain the well known atheromatosis of infancy.<sup>81</sup>

Eventually it is to be expected that some of the new, refined methods for studying the fine structure of connective tissue recently reviewed by Gross<sup>72</sup> will be applicable to the problem of arteriosclerosis.

**Summary**—It appears clear that much is yet to be learned about the early lesions of atherosclerosis, the factors governing permeation of the intima of arteries by lipids and the metabolism of cholesterol and other lipids with particular reference to the physical chemistry of their colloidal state in the blood. It seems possible that the level of serum cholesterol or at least the colloidal state of cholesterol in the blood is related in some way to the pathogenesis of atherosclerosis but it is not clear that dietary cholesterol has much influence on the level of blood cholesterol. Meanwhile the popularity of cholesterol should not be allowed to lead to neglect of other pathogenetic factors. It is possible that alterations in the ground substance may be of much greater importance (See below and Chapter 28).

**Etiology of Atherosclerosis in Diabetes**—As noted above there can be no question that diabetics are prone to develop atherosclerosis at an earlier age and to a greater extent than other persons. The reasons for this situation are difficult to assess.

It was pointed out in earlier editions of this book that fluctuations in blood sugar concentration might aid in producing swelling of the intimal ground substance through changes in osmotic pressure. In examining biopsies of the skin of diabetic patients in this laboratory it has been found that in those with high blood sugar concentrations the connective tissue of the corium may be slightly edematous and basophilic, strongly resembling the change seen in the intimal ground substance in early atherosclerosis.

Unna<sup>82</sup>  
robable

(increase of the mucopolysaccharides)

Much attention has been given to the level of blood lipids particularly cholesterol as a possible etiological factor in the atherosclerosis of diabetes. As noted above, it is both affirmed and denied that a relationship exists between the level of the blood cholesterol and the incidence of atherosclerosis. Certainly the diabetic is sometimes subject to an elevation of blood lipids and various lesions such as xanthoma have been considered to be related to this elevation. However the evidence is conflicting in that frequently the most severe degrees of atherosclerosis may be found in patients with relatively mild diabetes. Also it is not clear that well controlled diabetics have any striking aberrations of the serum cholesterol.<sup>77-78</sup> Perhaps some of the recently described methods of doing fat tolerance tests<sup>79</sup> and chylomicron counts<sup>80-82</sup> may cast light on this phase of the subject.

Wilens<sup>83</sup> has pointed out that overweight is common in all the important conditions that are believed to be associated with the atherosclerotic process (e.g. diabetes, hypertension). He shows that there is a distinct correlation between obesity and the presence of atherosclerosis. His study like those of Keys<sup>40-42</sup> suggests that constitutional and other factors must be considered in any analysis of the relation between lipid metabolism and atherosclerosis.

Pomeranze and Hunkel<sup>84</sup> found about 50 per cent of 273 diabetics to have a total lipid level above 750 mg. per cent which they considered the upper limit of normal. Although cholesterol, phospholipid and neutral fat were all increased the increment was greatest for neutral fat. They found a general correlation between arteriosclerosis and high total lipid, high cholesterol or reduced phospholipid/cholesterol ratio. They concluded that determination of cholesterol levels alone could not be used as an indicator of total lipid levels. Also there was no evident relationship between severity or duration of the diabetes or its control, age, sex or weight of the patient or size of liver and the total lipid level.

Of particular interest is the surprising finding of Duff *et al.*<sup>44-46</sup> that alloxan diabetes tends to inhibit experimental cholesterol atherosclerosis in the rabbit. These investigators found that the factor most consistently associated with the inhibition seemed to be the presence in the sera of the diabetic animals of a greater amount of neutral fat in proportion to the level of cholesterol than in the normal controls and suggested that this may be due to mobilization of body fat connected with the diabetic state. Their findings would seem to emphasize again the necessity of considering the concentrations and interrelations of *all* the lipids of the blood instead of singling out one such as cholesterol.

Scarcely any arteriosclerosis has been described in animals in connection with experimental diabetes. Allen and Liss<sup>85</sup> report a dog diabetic for twelve years following partial pancreatectomy and controlled by diet and insulin. No vascular lesions were found at autopsy.



On the other hand Keys<sup>70a</sup> holds that there is no evidence that the estimation of these giant molecules has any special virtue beyond that of simple cholesterol measurements in predicting atherosclerosis or in estimating the activity of the arteriosclerotic process.

Finally, it should be re-emphasized that arterial disease should not be considered in terms of lipoids alone to the exclusion of other well recognized factors such as mechanical stress, infection, vascularity of the vessel wall, physico-chemical characteristics of the ground substance (see Chapter 28) etc. Saphir and Gore<sup>71</sup> have recently stressed the possible etiologic role of infection in the coronary disease of young people. The work of Meyer<sup>72</sup> and of Faber<sup>73</sup> suggests that the connective tissue changes in atherosclerosis may be independent of the lipoid deposits. Blumenthal, Lansing *et al*<sup>15-18</sup> hold that calcification of elastic tissue (elasto-calcinosis) is a normal accompaniment of the aging process and is also a prerequisite for the formation of intimal plaques. This would not of course explain the well known atheromatosis of infancy.<sup>74</sup>

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**Etiology of Atherosclerosis in Diabetes**—As noted above, there can be no question that diabetics are prone to develop atherosclerosis at an earlier age and to a greater extent than other persons. The reasons for this situation are difficult to assess.

It was pointed out in earlier editions of this book that fluctuations in blood sugar concentration might aid in producing swelling of the intimal ground substance through changes in osmotic pressure. In examining biopsies of the skin of diabetic patients in this laboratory, it has been found that in those with high blood sugar concentrations the connective tissue of the corium may be slightly edematous and basophilic, strongly resembling the change seen in the intimal ground substance in early atherosclerosis. This change in the collagen (or in the interfibrillar substance) is very similar to the swollen, basophilic form seen in many elderly persons, which Unna has called *collacin*. (See also Chapter 28 for discussion of the probable importance of the mucopolysaccharides.)

Much attention has been given to the level of blood lipids, particularly cholesterol, as a possible etiological factor in the atherosclerosis of diabetes. As noted above, it is both affirmed and denied that a relationship exists between the level of the blood cholesterol and the incidence of atherosclerosis. Certainly the diabetic is sometimes subject to an elevation of blood lipids and various lesions such as xanthoma have been considered to be related to this elevation. However, the evidence is conflicting in that frequently the most severe degrees of atherosclerosis may be found in patients with relatively mild diabetes. Also, it is not clear that well-controlled diabetics have any striking aberrations of the serum cholesterol.<sup>77</sup> Perhaps some of the recently described methods of doing fat tolerance tests<sup>78</sup> and chylomicron counts<sup>79-82</sup> may cast light on this phase of the subject.

Wilens<sup>40</sup> has pointed out that overweight is common in all the important conditions that are believed to be associated with the atherosclerotic process (e.g., diabetes, hypertension). He shows that there is a distinct correlation between obesity and the presence of atherosclerosis. His study, like those of Keys,<sup>48-52</sup> suggests that constitutional and other factors must be considered in any analysis of the relation between lipid metabolism and atherosclerosis.

Pomeranze and Kunkel<sup>18</sup> found about 50 per cent of 273 diabetics to have

esterol, or reduced phospholipid/cholesterol ratio. They concluded that determination of cholesterol levels alone could not be used as an indicator of total lipid levels. Also, there was no evident relationship between severity or duration of the diabetes or its control, age, sex, or weight of the patient or size of liver and the total lipid level.

Of particular interest is the surprising finding of Duff *et al.*<sup>44-46</sup> that alloxan diabetes tends to inhibit experimental cholesterol atherosclerosis in the rabbit. These investigators found that the factor most consistently associated with the inhibition seemed to be the presence in the sera of the diabetic animals of a greater amount of neutral fat in proportion to the level of cholesterol than in the normal controls and suggested that this may be due to "mobilization of body fat connected with the diabetic state." Their findings would seem to emphasize again the necessity of considering the concentrations and interrelations of all the lipids of the blood instead of singling out one, such as cholesterol.

Scarcely any arteriosclerosis has been described in animals in connection with experimental diabetes. Allen and Lisa<sup>38</sup> report a dog diabetic for twelve years following partial pancreatectomy and controlled by diet and insulin. No vascular lesions were found at autopsy.

On the other hand Keys<sup>70</sup> holds that there is no evidence that the estimation of these giant molecules has any special virtue beyond that of simple cholesterol measurements in predicting atherosclerosis or in estimating the activity of the arteriosclerotic process.

Finally it should be re-emphasized that arterial disease should not be considered in terms of lipoids alone to the exclusion of other well recognized factors such as mechanical stress, infection, vascularity of the vessel wall, physico-chemical characteristics of the ground substance (see Chapter 28) etc. Saphir and Gore<sup>71</sup> have recently stressed the possible etiologic role of infection in the coronary disease of young people. The work of Meyer<sup>72</sup> and of Faber<sup>73</sup> suggests that the connective tissue changes in atherosclerosis may be independent of the lipid deposits. Blumenthal, Lansing *et al*<sup>74, 75</sup> hold that calcification of elastic tissue (elasto-calcinosis) is a normal accompaniment of the aging process and is also a prerequisite for the formation of intimal plaques. This would not of course explain the well known atheromatosis of infancy.<sup>76</sup>

Eventually it is to be expected that some of the new, refined methods for studying the fine structure of connective tissue recently reviewed by Gross<sup>77</sup> will be applicable to the problem of arteriosclerosis.

**Summary** It appears clear that much is yet to be learned about the early lesions of atherosclerosis, the factors governing permeation of the intima of arteries by lipids and the metabolism of cholesterol and other lipids with particular reference to the physical chemistry of their colloidal state in the blood. It seems possible that the level of serum cholesterol or at least the colloidal state of cholesterol in the blood is related in some way to the pathogenesis of atherosclerosis but it is not clear that dietary cholesterol has much influence on the level of blood cholesterol. Meanwhile the popularity of cholesterol should not be allowed to lead to neglect of other pathogenetic factors. It is possible that alterations in the ground substance may be of much greater importance (See below and Chapter 28).

**Etiology of Atherosclerosis in Diabetes**—As noted above there can be no question that diabetics are prone to develop atherosclerosis at an earlier age and to a greater extent than other persons. The reasons for this situation are difficult to assess.

It was pointed out in earlier editions of this book that fluctuations in blood sugar concentration might aid in producing swelling of the intimal ground substance through changes in osmotic pressure. In examining biopsies of the skin of diabetic patients in this laboratory it has been found that in those with high blood sugar concentrations the connective tissue of the corium may be slightly edematous and basophilic, strongly resembling the change seen in the intimal ground substance in early atherosclerosis.

Much attention has been given to the level of blood lipids particularly cholesterol as a possible etiological factor in the atherosclerosis of diabetes. As noted above it is both affirmed and denied that a relationship exists between the level of the blood cholesterol and the incidence of atherosclerosis. Certainly the diabetic is sometimes subject to an elevation of blood lipids and various lesions such as xanthoma have been considered to be related to this elevation. However the evidence is conflicting in that frequently the most severe degrees of atherosclerosis may be found in patients with relatively mild diabetes. Also it is not clear that well controlled diabetics have any striking aberrations of the serum cholesterol.<sup>77, 78</sup> Perhaps some of the recently described methods of doing fat tolerance tests<sup>79</sup> and chylomicron counts<sup>80-82</sup> may cast light on this phase of the subject.

Wilens<sup>83</sup> has pointed out that overweight is common in all the important conditions that are believed to be associated with the atherosclerotic process (e.g. diabetes, hypertension). He shows that there is a distinct correlation between obesity and the presence of atherosclerosis. His study like those of Keys<sup>40-42</sup> suggests that constitutional and other factors must be considered in any analysis of the relation between lipid metabolism and atherosclerosis.

Pomeranze and Kunkel<sup>84</sup> found about 50 per cent of 273 diabetics to have a total lipid level above 750 mg per cent which they considered the upper limit of normal. Although cholesterol, phospholipid and neutral fat were all increased the increment was greatest for neutral fat. They found a general correlation between arteriosclerosis and high total lipid, high cholesterol or reduced phospholipid/cholesterol ratio. They concluded that determination of cholesterol levels alone could not be used as an indicator of total lipid levels. Also there was no evident relationship between severity or duration of the diabetes or its control, age, sex or weight of the patient or size of liver and the total lipid level.

Of particular interest is the surprising finding of Duff *et al.*<sup>81, 85</sup> that alloxan diabetes tends to inhibit experimental cholesterol atherosclerosis in the rabbit. These investigators found that the factor most consistently associated with the inhibition seemed to be the presence in the sera of the diabetic animals of a greater amount of neutral fat in proportion to the level of cholesterol than in the normal controls and suggested that this may be due to mobilization of body fat connected with the diabetic state. Their findings would seem to emphasize again the necessity of considering the concentrations and interrelations of *all* the lipids of the blood instead of singling out one such as cholesterol.

Scarcely any arteriosclerosis has been described in animals in connection with experimental diabetes. Allen and Lasa<sup>86</sup> report a dog diabetic for twelve years following partial pancreatectomy and controlled by diet and insulin. No vascular lesions were found at autopsy.



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## Chapter 10

### GANGRENE

GANGRENE is one of the most serious complications of diabetes both from the nature of the lesion itself and from the wide path it opens to septic invasion of the body. Among 859 fatal cases of diabetes 144 cases of gangrene developed. Twenty nine were accompanied by generalized sepsis and there were 6 cases of gas gangrene.

TABLE 29 — ONE HUNDRED FORTY FOUR CASES OF GANGRENE AMONG 859 DIABETIC AUTOPSIES

	Number	Coma	Generalized sepsis	Gas gangrene
All cases	144	13	29	6
Cases over 15 years duration	29	0	7	0
Duration unknown	4	0	0	1

Bell<sup>1</sup> from a study of autopsy protocols at the University of Minnesota concluded that the diabetic process intensifies and accelerates to an astonishing degree vascular changes which are present to a lesser extent as part of the aging process in the extremities of nondiabetics. He states: "If only the gangrene due to atherosclerosis is considered, then gangrene develops nearly forty times as frequently in diabetic as in nondiabetic subjects."

**Embolic Gangrene** — Gangrene due to embolic occlusion of the larger vessels is the same in diabetic as in nondiabetic persons. No. 438, an arm from a diabetic aged sixty-one years showed embolism and secondary thrombosis of the axillary artery. There was slight medial calcification but no intimal change.

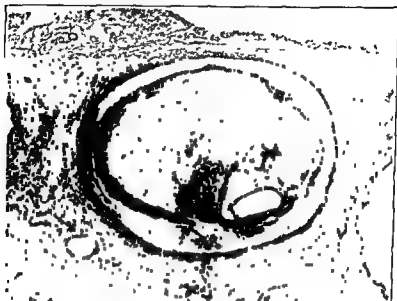
The youngest case of embolic gangrene in diabetes that we have seen occurred in Dr. Brigham's case.<sup>2</sup> Sudden gangrene of the left foot developed in a boy aged nineteen years who was in diabetic coma precipitated by pneumonia. In the article in which this case is reported 52 others have been collected from the literature.

**Gas Gangrene** — Only 6 cases of fatal gas gangrene have been encountered in 859 autopsies. Since the infectious feature of this condition is the most important it is discussed more fully in Chapter 8.

**Diabetic Gangrene** — It is frequently impossible to tell from the vessels alone whether a given specimen is from a diabetic or nondiabetic.<sup>3</sup> However, generally speaking the extent of involvement and especially the atheromatous thickening of the intima may be expected to be greater in



the diabetic Allen, Barker, and Hines<sup>1</sup> prefer the term "arteriosclerosis obliterans" for the type of vascular disease affecting the lower extremities. They state, however, that atheroma is the most important component of the lesion.



Duration of diabetes 43 years



FIG 67 — Lower portion of posterior tibial artery from same case as Figure 66 showing complete occlusion by intimal proliferation and only slight involvement of media, some calcification being present



There is one striking difference between arterio-sclerotic and diabetic gangrene—the former is more likely to be dry, the latter more commonly moist, although this distinction seems to be less marked since the introduction of antibiotics. The final occlusion of larger arteries is usually sud-

of a collateral circulation and anemic necrosis results. McKittrick and Root<sup>2</sup> illustrate excellently the absence of collateral circulation. As they point out, many diabetics show this type of gangrene.



FIG. 70.—Roentgenogram of injected leg after amputation. Case No. 27117, aged seventy years. Diabetic gangrene. Very extensive collateral circulation present. Note occlusion of dorsalis pedis artery. (McKittrick and Root<sup>2</sup>)



FIG. 71 —Early diabetic gangrene of foot and osteomyelitis. Case No. 37554. Aged seventy-four years. (McKusick and Root '9)



FIG. 72 —Gangrene due to embolic occlusion. (McKusick and Root '9)

Indbom<sup>6</sup> has published a beautiful arteriographic study of vascular disease in the extremities

Arterial occlusion in the typical diabetic gangrene is a gradual process at least at first—a progressive encroachment on the lumen of the artery by intimal thickening not infrequently showing heavy deposits of lipid. Hence there is time for collateral circulation to develop. This is well shown in Figure 70. While there may have been pain and disability during the process of readjustment eventually a delicate point of equilibrium is reached where the combined blood supply from both main vessels and collaterals is just sufficient for the ordinary needs of the limb. Any unusual stress will tip the balance. There will be insufficient blood supply to maintain life of the tissues under abnormal conditions too much to permit mummification. Moist gangrene results and all too often leads to generalized sepsis.

On this basis we may say that the typical diabetic gangrene is preventable. Injury and particularly infection can be prevented. Dirt ill fitting shoes carelessness all are simple all inexcusable in properly taught patients (and only the diabetic with knowledge of how to care for his special hazards and disabilities may defeat the disease) but nevertheless they cause many cases of gangrene.

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## Chapter 11

### THE HEART

A NATURAL sequel of the prevalence of arteriosclerosis in diabetic patients is the very frequent occurrence of myocardial injury due to coronary disease. A striking proportion of diabetic patients die of cardiac injury with or without coronary occlusion and infarction. While in the interpretation of the severity of chronic myocardial lesions the personal equation may enter (some individuals regard a given degree of fibrosis as more severe than others would) nevertheless there are four unequivocal lesions concerning the interpretation of which there can be no question. These are (1) a fresh infarct of the heart (2) a healed infarct of the heart with or without aneurysmal dilatation (3) rupture of the heart which is practically always due to infarction (4) coronary occlusion. As may be seen by reference to Table 37 in Chapter 19 these lesions are appallingly frequent in diabetic patients.

The statistics of infarction are widely variable and depend somewhat on the type of practice from which the material is drawn.



11-73—Heart showing healed and fresh infarcts. Note adherent thrombus in left ventricle. Female aged fifty-nine years. Duration of diabetes fifteen years.

(177)

Ophuls<sup>1</sup> in 3000 autopsies, of which 34 were on diabetic patients, found 18 cases of recent infarct and 8 of healed infarct. Seven cases showed aneurysms of the left ventricle and in 2 the aneurysmal sac had ruptured.

In 1000 consecutive unselected autopsies at the Mayo Clinic 49 infarctions were found.<sup>2</sup>

Clawson<sup>3</sup> in a survey of 30 265 autopsies, concluded that about 4 per cent of the deaths could be attributed to coronary sclerosis.

The occurrence of infarcts and coronary occlusion in the present series is summarized in Table 30.

TABLE 30 — INCIDENCE OF INFARCTS OF HEART AND CORONARY THROMBOSIS IN 761 DIABETIC AUTOPSIES

	Male				Coronary thrombosis without infarction
	Fresh	Healed	Fresh and healed	Total	
Number of cases	21	35	21	83	5
Average age years	61.5	60.3	60.2		59.0
Average duration years	14.3	12.5	10.6		10.7
Duration unknown	1	4			
	Female				
	Fresh	Healed	Fresh and healed	Total	
Number of cases	45	24	26	95	10
Average age years	61.2	63.3	63.9		61.8
Average duration years	10.8	13.5	11.0		11.0

Thus 178 cardiac infarctions (24 per cent) and 15 additional coronary thromboses without infarction were found in 761 diabetic autopsies.

Forty-seven cases had weathered one or more myocardial infarcts before the one that proved promptly fatal. On the other hand 15 cases died of coronary thrombosis without time for the development of demonstrable myocardial infarctions.

That diabetes is not merely an incidental feature in these cases is illustrated by the duration of the diabetic process, long antedating the cardiac condition. The average duration is twelve years, thus giving ample time for the abnormal metabolic conditions to affect the vessels.

Reference to Table 37 in Chapter 19 shows that fully 43 per cent of the deaths in these long duration cases were directly attributable to disease of the coronary arteries. Many others, of course, had severe coronary disease.

In our series of the 178 infarcts of the heart 54 per cent occurred in females.

Nathanson<sup>4</sup> in reviewing 100 diabetic autopsies found extensive coronary disease in 52 per cent of diabetics above fifty years of age, as against 8 per cent in general autopsy material.

Root, Bland, Gordon, and White<sup>5</sup> compared 349 diabetic autopsies with 3400 nondiabetic autopsies and found coronary occlusion to be much more

frequent in the diabetics and to occur almost as frequently in diabetic women as in diabetic men.

Stearns, Schlesinger and Rudy<sup>6</sup> found significant coronary disease in approximately three fourths of 50 diabetic hearts studied by Schlesinger's injection technique. Among diabetic women over forty they found coronary artery disease to be as common as among diabetic men. This meant that in their diabetic series coronary occlusion increased twofold in men and eightfold in women.

The most extensive study of the incidence of coronary artery disease in diabetes is that of Clawson and Bell.<sup>7</sup> They reviewed a series of 50,000 autopsies and found that fatal coronary disease was about twice as frequent in diabetic as in nondiabetic males and three times as frequent in diabetic as in nondiabetic females. They also concluded (in common with other writers) that the incidence of disease of the coronary arteries in diabetic women is almost as high as it is in diabetic men, in contrast to the marked preponderance of the disease in nondiabetic men over nondiabetic women. In their series about 4 per cent of the deaths due to coronary disease in males and 14 per cent of those in females were associated with diabetes.

Millard and Root<sup>8</sup> found that 89 per cent of a series of 106 diabetics showed coronary sclerosis of some degree.

Liebow and Hellerstein<sup>9</sup> summarizing several papers from the literature record figures for the post-mortem incidence of marked coronary sclerosis in nondiabetics ranging from 8.2 to 37 per cent and for diabetics from 45 to 74 per cent. Obviously differences in the observers' judgments as to the presence of marked or severe coronary disease must account for a good deal of the variation in these figures. The same authors present another table showing the post-mortem incidence of coronary thrombosis varying according to different reports from 2 to 23 per cent for nondiabetics and from 19.6 to 64 per cent for diabetics. Here again observational differences must be important and it is noteworthy that the high figure of 64 per cent was obtained by use of Schlesinger's injection technique which is recognized as disclosing a higher percentage of coronary occlusions than any other method (see Fig. 78).

One of the youngest deaths from cardiac infarction included in our series of autopsies was a patient of Dr. Joslin, Case No. 1794. When she was twenty-five years of age diabetes developed. Under insulin treatment she had permitted her weight to increase from 67 to 135 pounds. At the age of thirty-three years, eight years after the onset of diabetes, she developed intense precordial pain after breaking diet. Death occurred in a few hours after several Cheyne-Stokes attacks. At autopsy she showed fresh infarction of the apex of the left ventricle and the interventricular septum. The left coronary artery was practically completely occluded, barely admitting a horsehair in its first portion. The right coronary artery showed numerous atheromatous plaques. Both of the coronary orifices were surrounded by





years had been kept on a low-carbohydrate high fat diet. Death was due to coronary sclerosis and occlusion associated with widespread atherosclerosis.<sup>11</sup>

Diabetes is by no means a prerequisite for coronary disease in youth as is shown in the remarkable series reported by Yater *et al*<sup>12</sup> of coronary occlusion in young men in military service. Here of course the diabetics were screened out before induction.

When we consider chronic myocardial fibrosis criteria are difficult to establish. However scattered diffuse scarring of the musculature chiefly perivascular and barely visible in gross was considered slight. Possibly these fine scars might be regarded as physiological rather than pathological. They are encountered very frequently in the hearts of elderly persons non-diabetics as well as diabetics. A moderate degree of chronic myocardial fibrosis was diagnosed when fine diffuse scarring was visible to the naked eye and when the microscope revealed frequent small foci of fibrosis. The presence of generalized fibrosis both gross and microscopic was considered as evidence of a marked degree of myocardial damage.

Fairly closely paralleling these changes so far as severity is concerned and of course underlying them are the changes in the coronary arteries. Here again three grades of involvement are considered:

- 1 Slight scattered atheromata or fibrous thickening of the intima with but slight narrowing of the lumen
- 2 Moderate scattered atheromata with or without calcification or fibrous thickening of the intima the lumen reduced not over one-third
- 3 Marked numerous atheromata usually with some calcification or fibrous thickening of the intima the lumen reduced more than one-third. Actual occlusion may or may not be present.

TABLE 31 — MYOCARDIAL SCARRING AND CORONARY DISEASE IN 841  
DIABETIC AUTOPSIES

	Myocardial fibrosis	Per cent	Coronary sclerosis	Per cent
None	33	45.1	231	27.8
Slight	160	19.0	169	20.1
Moderate	98	11.6	141	17.0
Marked	146	17.4	261	31.1
Total	787	93.1	813	96.7
Data missing	54	6.9	28	3.3

Clinically angina pectoris is abnormally frequent in diabetic patients. The sex ratio is nearly equal. Since angina occurs in young diabetics since the duration of diabetes prior to angina is usually long and since the prognosis is poorer for diabetic patients diabetes itself is in all probability a definite factor in the disease.<sup>13</sup>

The relatively short duration of life after the onset of angina pectoris in a diabetic is well explained by the severity of the coronary disease and myocardial change found at autopsy.

The occurrence of hypertrophy of the heart is not infrequent, as is shown by Table 32

TABLE 32 — WEIGHTS OF THE HEART IN 817 DIABETIC AUTOPSIES

	Number	Per cent
0 to 350 gm	428	52.4
351 to 500 gm	299	36.6
500+ gm	90	11.0

Three hundred and fifty grams was considered as the upper limit of the normal heart. Those weighing over 500 grams were considered markedly hypertrophic.



FIG. 75 — Roentgenogram of heart from case of infarction and coronary thrombosis No. 5142. Male, aged sixty-one years. Duration of diabetes one and a half years. Note the marked calcification of the coronary vessels, also the hypertrophy of the heart. The weight was 520 gm.

In the coronary arteries as in other arteries of the diabetic patient intimal changes of the atheromatous type predominate. The involvement is not uniform, an artery may be almost completely occluded at one point and relatively normal at another. As elsewhere the atheromatous deposits tend to become calcified and the arteries may become of pipe-stem character or show spots of calcification. Roentgen ray examination of the excised heart demonstrates the calcification well as shown in figures 75 and 76. In the living body this method is not of value.



FIG. 76.—Roentgenogram of heart from Case No. 4796. Male, aged seventy six years. Duration of diabetes thirty years. Infarct of heart with rupture. Note calcification of right as well as left coronary arteries.

In addition to the atheromatous type of involvement fibrous thickening of the intima frequently occurs.

While the correlation between coronary arteriosclerosis and that elsewhere in the body is fairly close in the diabetic patient there are wide discrepancies at times, just as are found in nondiabetics.

The most satisfactory method for demonstration of coronary lesions is that of Schlesinger.<sup>14</sup> By the use of colored radio-opaque injection masses, both coronary occlusions and anastomoses can be brought out and the

## THE HEART

details subsequently studied by both dissection and histologic examination. Figure 78 shows the roentgenogram obtained from the heart of Case No. 37246, a woman aged sixty-four years, duration of diabetes twelve years. There is thrombotic occlusion of right coronary secondary to arterio sclerosis of atheromatous type, several small occlusions in right coronary distribution and the old arteriosclerotic occlusion of the left anterior descending branch. Note also the abundant anastomotic circulation. This heart showed no appreciable myocardial damage and illustrates how well collateral circulation may compensate for gradually developing occlusions.

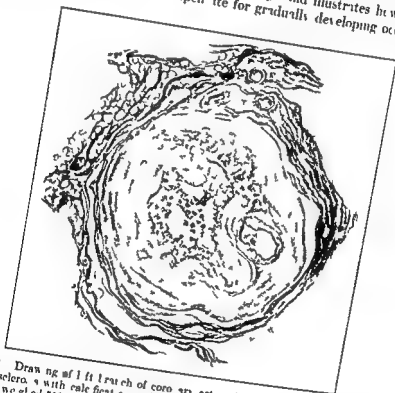


FIG. 78. Drawing of left branch of coronary artery from a heart showing marked coronary sclerosis with calcification and occlusion and a healed infarct of the heart. The organ weighed 510 gm. Note extreme involvement of intima. Apparatus in the center is due to large mass of fat and cholesterol crystals which have been dissolved out in the process of preparing the section. Male aged fifty-four years. Duration of diabetes 8 years. Hematoxylin-eosin stain.  $\times 24$ .

Another serious myocardial injury not infrequently encountered is that of multiple abscesses. In cases is prone to epistaxis those of diabetics it is not remarkable that we should find in this series 7 cases of abscesses of the myocardium.

Diffuse pericarditis was a cause of death in 7 cases and was accompanied by coronary in 2 other fatal cases. In 2 additional cases healed pericarditis of the chronic adhesive type was found. Thus 11 of the cases showed evidence of severe past or present pericardial inflammation.



FIG. 78 —Roentgenogram of heart injected by method of Schlesinger. Note occlusions and anastomoses. Female, aged sixty-four years. Duration of diabetes twelve years.

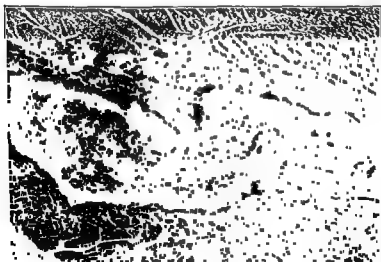


FIG. 79 —Large abscess of the heart from a case of carbuncle with *Staphylococcus aureus* septicemia and numerous abscesses involving heart, liver, and kidneys. Male, aged fifty years. Duration of diabetes twenty-three years.

In view of the marked susceptibility of diabetic patients to infection and the relative frequency of terminal septicemia the rarity of bacterial endocarditis and valvular lesions is very striking.

In Chapter 5 the increased amount of glycogen in the diabetic heart was discussed briefly and attention was drawn to the tendency of the glycogen to be deposited in particularly large amounts in the fibers about the margins of infarcted or fibrosed areas presumably indicating that the muscle fibers at this point were not able to utilize so fully as those elsewhere the amount of glycogen deposited in them. Glycogen apparently exerts no deleterious effect through its presence although some question may be raised about this point as regards the infants of diabetic mothers (see Chapter 23) and glycogen storage disease.

Landing and Bingle<sup>14, 15</sup> have discussed some of the difficulties encountered in making a histochemical distinction between the heart of glycogen storage disease and that of diabetes mellitus also the evidence suggesting an hereditary linkage between the two diseases. Mowry and Bingle<sup>16</sup> confirm the presence of large amounts of glycogen in diabetic hearts.

Reference has been made in Chapter 7 to the marked fall in serum potassium which may occur in diabetic acidosis. In evaluating the presence or absence of injury from either low or high potassium concentration the electrocardiogram has proven indispensable. In fact the electrocardiogram has been shown to be a more accurate index to disturbed potassium metabolism than the level of serum potassium is indeed might be expected from the fact that potassium is primarily an intracellular ion.

Rodriguez *et al.*<sup>17</sup> have described two cases of what they call hypokalemic myocarditis in diabetic coma. The lesions which consisted essentially of multiple small foci of necrosis together with extensive lymphocytic infiltration were regarded as due to potassium deficiency on the basis of similar lesions produced experimentally by other workers and a few other human cases.

An admirable review of the cardiac complications of diabetes including the functional effects of insulin of high and low potassium levels and the cardiovascular collapse often encountered in coma is given by Tiebout and Hellerstein.<sup>18</sup>

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## Chapter 12

### THE KIDNEY

The kidney of the diabetic patient may exhibit any of the disease processes found in nondiabetics. However, certain lesions are encountered with far greater frequency in diabetics and two of them (glycogen deposits in the tubules and intercapillary glomerulosclerosis) are almost pathognomonic of the disease.

The renal lesions which frequently complicate diabetes may be listed as follows:

1 Presumably reversible metabolic phenomena

a Glycogen deposits in tubules

b Fat deposits in tubules

2 Arteriolar sclerosis

3 Intercapillary glomerulosclerosis

4 Acute and chronic pyelonephritis including necrotizing renal papillitis

It should be emphasized at the outset that it is common for two or more of these lesions to be found together in the same kidney in the diabetic. This is particularly true of the last three diseases named and in a case of advanced renal disease it may be very difficult or impossible to assess their relative importance. The younger diabetics who have had their disease for many years almost invariably show a mixture of renal lesions so much so that one may speak of a mixed diabetic nephropathy as characterizing these patients (see Wilson *et al*<sup>1</sup> Adams<sup>2</sup> and Chapter 19).

Glycogen deposits in the renal tubules (so-called glycogen nephrosis) are discussed elsewhere (see Chapter 5). As noted there such deposits are presumed to be evidence of terminal glycosuria in a hypothesis which was put forth in earlier editions of this book and which has been given experimental confirmation by Curtis *et al*<sup>3</sup>. The lesion has no known clinical significance other than as an indicator of glycosuria. Experimentally, it can be shown to be reversible by lowering the blood sugar after previous high levels have been maintained.<sup>4</sup>

Fat deposits are also mentioned elsewhere (see Figure 55). Fat appears in the renal tubules under a variety of conditions<sup>5</sup> and is usually regarded as a reversible manifestation of injury to the renal epithelium. As noted in Chapter 7 it has been seen in the diabetic usually in association with acidosis and coma<sup>6</sup> which in the past was often further complicated by sepsis. Cases without recent coma may also show fat.

## THE KIDNEY

Recently Mallory<sup>7</sup> has shown that fat vacuolation of the ascending limb of Henle's loop is seen in the majority of cases of traumatic shock surviving one to four days, although he found it only rarely in cases surviving less than eighteen hours. Circulatory collapse with blood pressures of shock level is common in diabetic coma; it is probable however that the duration of shock in the usual case of coma is insufficient to account for the lesion. Also in the cases of outspoken fat deposition seen in this laboratory the vacuolation was by no means confined to Henle's loop (Figure 55).

Recently a striking case of fatty change of the renal epithelium was seen. The patient, a sixty-seven-year-old woman, had had diabetes for twenty-two years. She died after being in coma for thirty hours; the admission

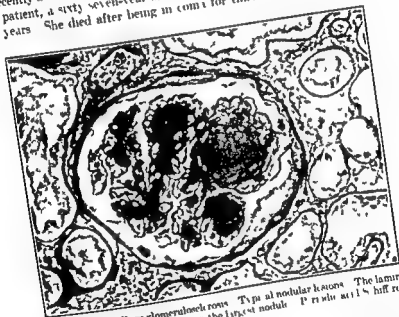


FIG. 80.—Intercapillary glomerulosclerosis. Typical nodular lesions. The lamination of the hyaline material is well shown in the largest nodule. Periodic acid-Schiff reagent method according to McManus.  $\times 250$ .

blood sugar having been 720 mg. per cent and the  $\text{CO}_2$  (after some treatment) 22 vols. per cent. The patient had a blood pressure in shock levels for several hours. The kidneys showed no striking damage to the glomeruli other than a few small foci of fibrosis. The epithelium of the convoluted tubules showed large vacuoles which were shown by frozen section and Sudan staining to be fat.

Interstitial sclerosis or benign nephrosclerosis is common in the diabetic kidney. Anatomically it takes the same form as in the nondiabetic as described by Moritz and Oldt<sup>8</sup> and by others. However in the diabetic it is likely to appear earlier and to be more severe even in cases with apparent good control of

some degree of vascular disease in

the kidney has been noted in a large proportion of the diabetic autopsies in this laboratory (see Chapter 19), and Robbins and Tucker<sup>10</sup> found arteriolar disease of the kidneys in 55 per cent of their diabetic series. Bell<sup>11</sup> found renal arteriosclerosis in 72 per cent of diabetics over fifty years of age and states that it is nearly as frequent in nonhypertensive as in hypertensive diabetics. He also found that it increases in frequency with the duration of diabetes in persons under fifty.

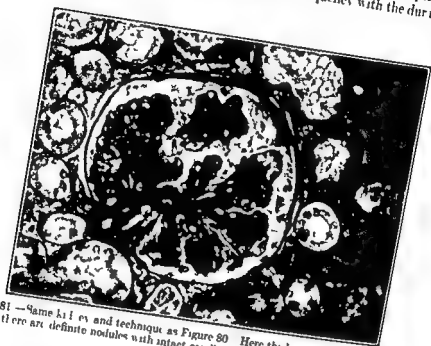


FIG. 81.—Same kidney and technique as Figure 80. Here the lesion is more diffuse but there are definite nodules with intact capillaries on their periphery.  $\times 250$ .

Death from renal insufficiency due to this lesion recorded is rare in the earlier editions of this book, is now becoming relatively common in diabetics who live longer with their disease. Accordingly arteriolar disease is also seen with increasing frequency in the younger diabetics who have had their disease for a number of years. In such cases the lesion is often part of a widespread involvement of arterioles throughout the body and exhibits itself with distressing results in the retina where it may be combined with capillary and venous lesions (see Chapter 13). In the kidney it is often seen in combination with either intercapillary glomerulosclerosis or pyelonephritis or both and may contribute to death from renal insufficiency, a relatively rare situation in the nondiabetic hypertensive patient.

**Intercapillary Glomerulosclerosis.**—This disease has excited increasing interest from both the histopathological and clinical points of view, since its description by Kimmelstiel and Wilson in 1936.<sup>12</sup> One of the original authors has participated in an excellent recent review.<sup>13</sup> As noted there

## THE KIDNEY

considerable confusion has arisen as to the histological definition of the lesion resulting in estimates of its incidence in diabetes varying from 20 per cent<sup>14</sup> to 63.7 per cent<sup>15</sup>

In its fully developed nodular form the lesion consists of one or more hyaline masses situated in the central part of the glomerular lobule creating the impression of being situated between the capillaries so that characteristically an intact capillary is seen on the margin of the hyaline mass (Figs 80 and 81). The focal character of the lesion and the presence of intact capillary loops in the involved glomerulus are important diagnostic features. Such a histological picture must be clearly distinguished from similar but more diffuse changes which may occur in chronic glomerulonephritis<sup>16</sup>. The lesion is usually associated with arteriosclerosis of the afferent or efferent arterioles or both. Allen<sup>17</sup> considers that sclerosis of the efferent arteriole is significant since he found it only in his diabetic cases. Bell's statement<sup>18</sup> that intercapillary glomerulosclerosis is merely a severe form of arteriosclerosis with extension into the glomeruli seems to be perhaps an oversimplification of the problem. (See Allen<sup>17</sup>.)

The hyaline nodule may resemble amyloid with routine histological methods but it does not have the staining reactions of amyloid with the exception of an occasional positive reaction with Congo red. With silver stains with McGregor's modification of Mallory's aniline blue stain and with the periodic acid method of McManus<sup>19</sup> it may sometimes be shown to have a laminated appearance (Fig 80). Frozen sections may show variable amounts of lipid. Allen<sup>20</sup> also found this type of hyalin to be more resistant to tryptic digestion than nondiabetic hyalin. McManus<sup>19</sup> found that it was removed by pectinase from acetone fixed tissue.

The literature contains considerable dispute regarding the histogenesis of the process. Chief disagreement centers around whether the initial lesion is truly intercapillary. Allen<sup>21</sup>, Bell<sup>22</sup> and Iuppi<sup>et al.</sup><sup>23</sup> contend that the change arises by a splitting of the capillary basement membrane and Allen would apparently prefer to think of the process as intramural. McManus<sup>24</sup> holds that the hyaline material is deposited in the intercapillary space of Zimmernum. Much of the difference of opinion depends upon whether one accepts the presence of intercapillary connective tissue at the periphery of the normal glomerular lobule. Bell<sup>25</sup> appears to deny its existence but Kimmelstiel and Porter<sup>26</sup> quote Zimmernum as having convincingly demonstrated its presence and at the same time suggest that it may be impossible in any given section to determine how far out into the lobule the intercapillary tissue extends. Iahr<sup>27</sup> drawing an analogy between the structure of the glomerular tuft and the tubule. Kimmelstiel and Porter<sup>26</sup> discuss as extracapillary glomerulosclerosis. Kimmelstiel and Porter<sup>26</sup> point out that the concepts of Allen, Bell<sup>22</sup> and Iahr represent differences in interpretation not of observation and suggest that the term intercapillary glomerulosclerosis be retained since it serves well for descriptive identification.

Bell<sup>14</sup> has made what appears to be a useful distinction in describing two forms of the disease namely, nodular and diffuse. The nodular type is the classical picture described above. The one most easily recognized and most characteristic of diabetes is the diffuse form which is not clearly delineated consists in a diffuse thickening of the capillary basement membrane formed by splitting of said membrane. According to Bell<sup>14</sup> it may occur alone or together with the nodular form. His series showed an incidence of the nodular lesion in 15.9 per cent of diabetics and the combined nodular and diffuse in 29.2 per cent. As noted by Kimmelstiel and Porter<sup>15</sup> the diffuse type would seem to be susceptible of varying interpretations. It seems likely that this accounts largely for the extraordinary variation in reported incidence of intercapillary glomerulosclerosis in diabetes. For instance the remarkably high figure of 63.7 per cent given by Laipply *et al*<sup>16</sup> is accounted for by their inclusion of one plus lesions characterized by focal fibrosis of the majority of glomeruli.

In unpublished studies in this laboratory,<sup>17</sup> attempts have been made to confirm the findings of Laipply *et al* with the aid of a few slides kindly sent by Dr Laipply for comparison. After study of various types of glomerular change in nondiabetics as well as diabetics it was concluded that it was very difficult to be certain of the specificity of the minimal changes described by Laipply *et al*, as representing the early stage of the process. Our own estimate of the incidence of the disease in diabetics approximates the figure of 17 per cent arrived at by Kimmelstiel and Porter<sup>15</sup> from a careful survey of the literature. This figure would certainly be higher if the disease were not as noted above so commonly complicated by arteriolar nephrosclerosis or pyelonephritis or both in which cases the diagnosis must often remain in doubt. Also when one considers only the younger patients who have had their disease for many years the incidence mounts to a very high figure (*see below*).

It appears likely that the nodular form of the disease is practically specific for diabetes although it is possible that rare cases have been observed in nondiabetics. In such cases it is not always clear that diabetes has been positively excluded by an adequately prolonged period of observation, glucose tolerance tests and the like. McManus<sup>20</sup> notes that glycosuria and even hyperglycemia may disappear with the onset of renal insufficiency in intercapillary glomerulosclerosis. (See also Lubrod *et al*<sup>21</sup>).

Kimmelstiel and Porter<sup>15</sup> agree with Henderson *et al*<sup>22</sup> that certain cases of chronic glomerulonephritis may show lesions indistinguishable from intercapillary glomerulosclerosis. The latter authors have presented a comparative clinical study emphasizing certain points of difference between the two diseases.

Robbins and Rogers<sup>23</sup> have reviewed 100 proven cases of intercapillary glomerulosclerosis and 229 cases of diabetes. They concluded that 22.4 per cent of the cases of glomerulosclerosis had diabetes.

## THE KIDNEY

diabetes in

of the nodular type of intercapillary glomerulosclerosis. The lesion is a specific discrete histologic entity occurring only in diabetes and chronic glomerulonephritis and conclude that in the nodular form the lesion is a specific discrete histologic entity occurring only in diabetes.

Experimental reproduction of the lesion was achieved by Lukens and Dohan<sup>24</sup> in a single dog which had been maintained in a diabetic state for five years by administration of an extract of the anterior lobe of the pituitary gland. Their illustration of the lesion appears convincing. More recently Mann and Goddard<sup>25</sup> have found in rats made diabetic with alloxan a lesion similar to though by no means identical with the human disease. The earliest sign is described by them as a thickening of the basement membrane followed by deposition of hyaline acellular material between the capillary loops with in contrast to the human lesion eventual adhesions of the tufts to one another or to Bowman's capsule. There is also again in contrast to the human lesion proliferative activity on the part of endothelial and epithelial cells in the glomerulus.

The lesions described by Mann and Goddard<sup>25</sup> are probably essentially similar to those described by Loggia *et al.*<sup>26</sup> in rats made diabetic by partial pancreatectomy. In neither case can the experimental disease be regarded as a reproduction of the human lesion.

It seems generally agreed that the incidence of the lesion is more closely related to the duration than to the severity of the diabetes and our own observations conform to this opinion. Some authors<sup>1, 2, 7</sup> find a correlation between duration of diabetes and incidence and severity of the lesion others<sup>3</sup> do not.

There seems to be a wide spread and we think erroneous impression that intercapillary glomerulosclerosis is rare in young diabetics. On the contrary it appears to be common at least in those of long duration. (See also Dawson and Platt<sup>27</sup>.) In a small series of young diabetics of long duration observed in this laboratory intercapillary glomerulosclerosis appears to be present in almost 100 per cent the few in which it cannot be clearly recognized representing cases in which it may be obscured by other severe lesions as noted above. Indeed renal disease has become a major problem in these young patients (see also Millard and Root<sup>28</sup> Mann, Gardiner and Root<sup>29</sup> Wilson *et al.*<sup>3</sup> and Adams<sup>30</sup>). According to this finding one might consider the renal lesion as related to the severity of the diabetes since the younger patients often have the more severe diabetes. However many years seem to be required before the renal disease is manifest in these cases.

The time required for development of the lesion is unknown. Apparently it may appear fairly rapidly as indicated by the interesting case of Derow and Schlegler.<sup>31</sup> Their patient a sixty-one-year-old woman had had

diabetes for eight years before her death. Four years before death a nephrectomy had been done. Careful study of sections of this kidney revealed only a single nodular lesion in a single glomerulus which the authors are inclined to discount because of its lack of association with intimal thickening of the glomerular tuft. Two years before death the patient developed massive albuminuria and at autopsy showed a severe nodular form of intercapillary glomerulosclerosis in the remaining kidney. The authors suggest that the patient may have had the lesion for only two years coincident with the albuminuria.

There appears to be no correlation between intercapillary glomerulosclerosis and hyalinization of the islets of Langerhans in the pancreas. However a striking correlation with retinal changes has been noted. Henderson *et al*<sup>23</sup> found retinopathy in 68.8 per cent of cases of intercapillary glomerulosclerosis as opposed to 22.8 per cent in cases of diabetes without intercapillary glomerulosclerosis. Dolger<sup>24</sup> found that retinopathy tended to appear approximately thirteen years after onset of diabetes whether in the third or fourth decade and to be associated in 81 per cent of cases with hypertension and albuminuria most of the latter being presumed to have intercapillary glomerulosclerosis. Studies of this sort incited Kimmelstiel and Porter<sup>25</sup> to suggest that retinopathy of the diabetic type should be considered part of the clinical symptom complex associated with the renal disease.

The clinical recognition of intercapillary glomerulosclerosis is perhaps more difficult than was thought in the first few years of study of the disease. Robbins and Rogers<sup>16</sup> conclude that not more than 40 per cent of cases can be correctly diagnosed on clinical grounds. Contrary to earlier expectations it appears that a fully developed nephrotic syndrome can be expected in only about 10 per cent of cases. Hypertension another feature stressed in the first case reports is absent in as many as 40 per cent of some series (Henderson *et al*<sup>23</sup>). The diagnostic value of finding anti streptolysin in the urine is stressed by Riskin *et al*<sup>26</sup>.

Kimmelstiel and Porter<sup>25</sup> sum up the clinical findings as follows. With the exception of chronic glomerulonephritis it can be stated that diabetes occurs in almost all cases of intercapillary glomerulosclerosis. Hypertension is found in approximately sixty per cent. Significant albuminuria is found in the majority of cases (approximately two thirds) edema of the

that by correlating several of the above findings with patient's age a fairly accurate diagnosis may be made in some cases. One can say however that in a patient fifty or more years of age with chronic diabetes nephrotic edema and albumin in the urine with high blood cholesterol one can safely make the diagnosis of intercapillary glomerulosclerosis with approximately 100 per cent accuracy. They add that

glomerulonephritis may be excluded by repeated Addis counts which may be expected to reveal hematuria not found in intercapillary glomerulosclerosis.

The above statement should perhaps be modified to the extent that intercapillary glomerulosclerosis is probably more closely correlated with duration of diabetes than with the age of the patient. As noted elsewhere the incidence of intercapillary glomerulosclerosis in young diabetics of long duration is extraordinarily high both on clinical and pathologic grounds. Rather sudden onset of marked albuminuria together with rise in serum cholesterol is often noted in these patients.<sup>20</sup>

The pathogenesis of the disease remains obscure. Prolonged diabetes appears to be the *sine qua non*. Hypertension although commonly found in association with it cannot be held accountable since undoubted cases occur in the absence of hypertension. Recently Wilens *et al.* have made a study of frozen sections stained with Sudan IV in 21 cases of the disease and find a statistically significant larger amount of fat in the glomeruli in this condition than in any of the other main varieties of renal disease in diabetes alone or in diabetic hypertensive patients without intercapillary glomerulosclerosis. Although in a few instances they found large amounts of glomerular lipid in other types of renal disease the deposition of fat in these conditions bore no constant relation to the severity of the renal lesions. In intercapillary glomerulosclerosis however the quantity of glomerular fat was directly proportional to the severity of the lesions in most instances. From these data and from the distribution of the lipids within the hyaline masses and between the glomerular tufts they conclude that deposition of fat in the glomeruli may be of primary importance in the development of the lesions of the disease. We have confirmed the presence of lipid droplets in the glomeruli but have not made a quantitative study of the phenomenon.

The possible etiologic relation of the lesions of intercapillary glomerulosclerosis to the capillary aneurysms of the retina especially in relation to the polysaccharide content of the lesions forms a fascinating subject for speculation (see Chapters 13 and 28).

**Pyelonephritis.**—The frequency of urinary infection in diabetes is well recognized.<sup>21</sup> In a series of 196 autopsied cases Sharkey and Root<sup>22</sup> found purulent infection of some part of the urinary tract in 18 per cent. Robbins and Tucker<sup>23</sup> found acute pyelonephritis to be four and a half times more common as a cause of death among diabetics than in nondiabetics. In their series of 307 diabetic patients it was the sixth commonest cause of death causing 21 of the fatalities. They found some evidence of acute pyelonephritis in 1 out of every 5 autopsies on diabetics.

This conforms to experience in this laboratory. Even now when acute pyelonephritis is being controlled more and more with the antibiotics evidence of healed lesions corresponding to the classical description of Weiss and Parker<sup>24</sup> is found with great frequency.



Recently a particular type of acute pyelonephritis known as *necrotizing renal papillitis* has received increasing attention. In the series of 307 cases of diabetes reported by Robbins and Tucker<sup>40</sup> it occurred once in every four cases of acute pyelonephritis and was the severest form of the disease accounting for 16 of the 21 deaths in that group.

The lesion has been recognized for years, but has recently been the subject of three detailed reports<sup>39, 40, 41</sup> which emphasize clinical as well as pathological features.

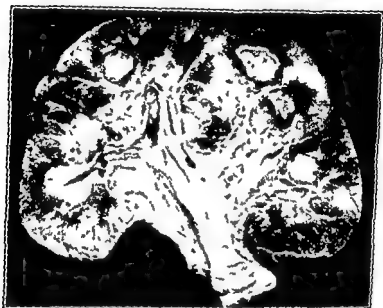


FIG. 82.—Necrotizing renal papillitis showing the infarct-like patches of necrosis in the pyramids. Photograph by courtesy of Dr. Stanley Robbins.

junction of the pyramid and the cortex. As the lesion progresses these abscesses become confluent, resulting in a complete necrosis of the terminal two thirds of the pyramid. Grossly the lesion resembles an infarct of the cortex but is likely to be yellow brown or yellow green with a purplish or purplish zone which in turn is often surrounded by a red congested border. The more usual type of acute pyelonephritis may be found in the cortex in varying degree. The process is frequently but not always bilateral and in most cases all the pyramids of the affected kidney are involved (Fig. 82). Eventually portions of the papillae may break off or sequestration of the whole papilla into the pelvis may occur with associated hematuria and

renal colic (Fig 83). A single case of an apparently healed lesion is described by Edmondson *et al*<sup>40</sup>.

The pathogenesis of the lesion remains obscure. While common in diabetics it is seen also in nondiabetics with obstructive lesions of the urinary tract. No particular bacteria are constantly found although *E. coli* and *Staphylococcus aureus* are frequent. The infarct like necrosis is thought to be associated with impairment of the already rather feeble capillary blood supply of the papillae. Small hyaline thrombi are commonly seen in these vessels.



FIG 83—Necrotizing renal papillitis. Low power. The necrotic tissue of the papilla has separated like a sequestrum.

A somewhat similar lesion was produced experimentally by Mallory<sup>41</sup> by ligation of one ureter in rabbits and intravenous injection of bacteria. No significant lesions appeared in the nonobstructed kidney, but on the obstructed side an acute pyelonephritis with necrosis of papillae was observed. Muirhead *et al*<sup>42</sup> produced something similar by aseptic ureteral ligation in dogs. However, they offer no evidence to explain the high incidence in diabetes without obstruction. Robbins and Angrist<sup>43</sup> call attention to the earlier work of Borland and Jackson<sup>44</sup> who described necrosis of the renal pyramids with calcification in rats on a fat free diet. The relation of this work, if any, to the human lesion is not clear. The same applies to the experimental medullary necrosis induced with vinylamine by Mandel and Popper<sup>45</sup>.

Clinically, the disease carries a high mortality and should be recognized as early as possible and treated vigorously. Edmondson *et al*<sup>40</sup> suggest that the disease should be suspected in the following types of diabetic patients: (1) those with sepsis in whom urinary symptoms or findings suddenly develop; (2) those who go into coma rapidly with nitrogen retention but without an antecedent history of pyelonephritis; (3) those with low-grade pyelonephritis who suddenly become worse; (4) those with hematuria or renal colic; (5) those who fail to rally from severe acidosis with ade-



FIG. 84.—Mixed diabetic nephropathy in a young diabetic of long duration. There is evidence of intercapillary glomerulosclerosis, arteriolar sclerosis and pyelonephritis.  $\times 150$ .

quate treatment and whose nonprotein nitrogen continues to rise even after the blood sugar and carbon dioxide have returned to normal and (ii) those with characteristic roentgenographic findings.

Robbins *et al*<sup>41</sup> emphasize that the disease may appear as an acute fulminating febrile illness without premonitory signs or else as an acute severe exacerbation of a chronic urinary infection.

The pyelograms may be diagnostic. The following changes have been emphasized:<sup>42</sup> (1) a deformity of the tip of a calyx similar to the usual



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## Chapter 13

### THE EYE

THE question as to whether the diabetic eye shows any characteristic or pathognomonic changes is still disputed. However certain lesions do occur more frequently in diabetics than in the general population. Some of these lesions are better studied with the slit lamp or ophthalmoscope than by histological techniques and have been particularly well summarized by White and Beetham<sup>1</sup> to whose paper the reader is also referred for a discussion of the functional alterations.

**Eyelids**—White and Beetham<sup>1</sup> reported a slightly increased incidence of xanthelasma\* and of squamous blepharitis in their series as compared with normal controls.

**Conjunctiva**—White and Beetham<sup>1</sup> found a slightly increased incidence of conjunctivitis in the diabetic series. Cultures in 40 cases showed a predominance of *Staphylococcus aureus* in most instances.

**Cornea**—In the series referred to<sup>1</sup> wrinkles of Descemet's membrane were found to be definitely more frequent in the diabetics. They also appeared at an earlier age than in the controls. The incidence of arcus was the same in both groups.

**Iris**—It is an old observation that in the operation for iridectomy in diabetic patients, iris pigment is readily set free and makes the aqueous dark and cloudy or alternatively in an operation for cataract a part of the pigment layer may come away with the lens.<sup>2</sup>

The cause of this phenomenon known for many years to ophthalmic surgeons is not clear. It is generally assumed that the pigment is released from the pigment layer of the iris and that the released pigment is then taken up by the vacuolated cells. He stated that the normal eye is free from glycogen but that in inflammation, circulatory disturbances and diabetes deposits of glycogen may be found in the iris, the corneal epithelium, the retina and the optic nerves. The presence of vacuoles and glycogen in the pigment layer of the iris in diabetes has been confirmed by others and Villam<sup>3</sup> has produced similar changes in rabbits given intraperitoneal injections of glucose over a period of twelve to thirty-four days. Although the presence of glycogen may not account entirely for the swelling of the pigment epi-

\* Xanthelasma is a yellowish deposit of cholesterol in the skin around the eyes.

thelium and the depigmentation of the iris frequently seen in diabetics it seems likely that it is a major factor

**Lens** The generally accepted manifestation of ocular pathology in diabetes mellitus is the prevalence of cataract. Noted first by Rolleston a hundred and forty years ago it is mentioned by practically all textbooks of ophthalmology. Only in recent years has there been some doubt as to whether cataract in the elderly person varies in the diabetic and in the nondiabetic. There is definite evidence that cataract in the juvenile is far more frequent in diabetic patients than in nondiabetic patients.

Witte and Beetham<sup>1</sup> in their careful study of ocular pathology in diabetes examined 2459 patients diabetic and nondiabetic. They found in

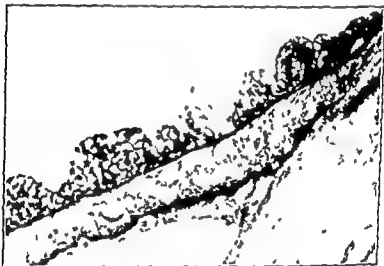


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those over twenty years old that a total of 50 per cent of the diabetic patients (3047 eyes) showed lens opacities of all types whereas among 526 eyes of nondiabetics 57 per cent showed opacities. Not only was the total number of lens opacities the same in diabetics and nondiabetics but there was virtually no difference in the proportion of complicated cataract found 6 per cent among 4001 diabetic eyes and 8 per cent among 914 nondiabetic eyes.

On the basis of Danish statistics Anthomisen<sup>2</sup> found that depending upon age from 15 to 202 times the number of cataracts in diabetics were operated upon in the Rigshospital as would be expected from the prevalence of cataract in the general population.

In a study of the chemical constitution of the cataractous lens of adult type in both nondiabetics and diabetics the findings as given by Carey and Hunt<sup>10</sup> are approximately the same. Thus in 30 nondiabetic and 13 diabetic cataractous lenses the cholesterol content was 525 and 511 mg per cent respectively, the calcium 30 and 35 mg per cent respectively. However, there was a distinct difference in the phosphorus content, there being in the nondiabetic 19.7 mg per cent of nonlipoid phosphorus as against only 6.2 mg per cent in the diabetic. The cholesterol content of the cataractous lens is approximately 20 per cent greater than that of the normal lens and the calcium content, roughly three times that of normal lens or normal blood.

Cataract has been a striking and frequent complication of experimental alloxan diabetes in rabbits and rats. (See Chapter 25.)

Waters<sup>11</sup> studied the biochemical changes in the lens of the eye during experimental diabetes in rabbits and found that although the glutathione content of the lens remained within normal limits, the total sulfhydryl content decreased within four hours after alloxan and remained low as long as two years. He concluded that the decrease must be due to a decrease in the sulfhydryl groups of proteins. Not all these rabbits got cataract, but the experiment showed that early chemical changes occur in the lens within a short time after alloxan.

One feature of cataract in the diabetic patient is seen in the juvenile patient where there is development of a distinctive type of opacity, distinctive in the sense that it does not occur in normal individuals although it is indistinguishable from the cataracts induced by various of the metabolic disorders and dinitrophenol. These opacities occur in the anterior and posterior cortical portions of the lens and resemble a chemical precipitate of flocculent nature. They have been described as diabetic cataracts by Schnyder<sup>12</sup> and as snowflake opacities by O'Brien, Molsberry, and Allen.<sup>13</sup>

An extensive discussion of cataract may be found in the monograph by Bellows.<sup>1</sup>

**Retina.** For many years the question as to whether a specific diabetic retinopathy exists has been a matter for debate among ophthalmologists.<sup>14</sup> Recently the opinion seems to be growing that in many diabetics, including some without hypertension and without evidence of arterial or arteriolar disease, there occurs an ophthalmoscopic picture characterized by small punctate and irregular hemorrhages, often associated with dilatation and tortuosity of the retinal veins with sharply defined, hard white exudates, and sometimes ending in the late stages in a type of retinitis proliferans in which many newly formed vessels are found. Several recent writers have laid particular stress on the small punctate hemorrhages (many of which are probably microaneurysms) as representing the earliest detectable lesion in diabetic retinopathy. As a result of this increased attention has been devoted to the study of the capillaries and venules rather than the arterioles as the probable site of the early damage in diabetes. This point was



emphasized by Waite and Beetham<sup>1</sup> who found deep retinal hemorrhages in 18 per cent of 1915 visible fundi in diabetics. Flynn<sup>15</sup> postulated a dilatation of the capillaries with consequent slowing of blood flow (which he called "prethrombosis") leading to diapedesis of erythrocytes and the formation of small hemorrhages. He felt that this condition was more closely related to hyperglycemia than to any other factor.

Particular interest attaches to the work of Ballantyne<sup>16, 17</sup> who described microaneurysms of the retinal capillaries as the earliest lesion in diabetic retinopathy. \* These aneurysms are described as about 50 to 60 microns in diameter, occur chiefly in the perimacular region, and are perfectly round. In fact many of the so-called punctate hemorrhages probably actually represent this lesion. Ballantyne describes them also in slit unstained

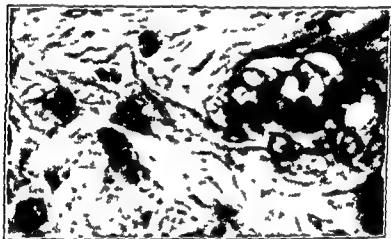


FIG. 86.—Diabetic retinopathy. The capillary passing from the left and slightly downward is dilated and filled out to a microaneurysm containing red blood cells.  $\times 1000$ .

specimens of the formalin fixed retina and in histological section. He states that they may have thickened hyaline walls and may show endothelial proliferation. Thrombosis and deposition of fat in the endothelium may also occur. We have found it difficult to locate these lesions by gross examination of autopsy specimens by Ballantyne's method, presumably because the retinal vessels in the usual cadaver are fairly well emptied of blood. However, in occasional fortunate sections we have encountered lesions which seem to conform to his description (Fig. 86).

The study of these capillary aneurysms has been greatly altered by Friedenwald's<sup>18, 19</sup> useful discovery of the value of the periodic acid Schiff reagent method of Hotchkiss and McManus when applied to whole mounts.

\* As noted by Sierrall<sup>20</sup> retinal microaneurysms were described and illustrated by Edward Nettlehip in a case of glycosuric retinitis reported by Mackenzie in 1879.

of the retina. By this method, which has also proved so valuable in studying the renal glomerulus (see Figs. 80-81), the capillary basement membranes are stained in intense purple red. When the whole retina is stained, dehydrated, cleared, and mounted flat without sectioning, a beautiful delineation of the capillary network is obtained, and it becomes clear that the lesions described by Ballantyne are indeed aneurysms, which often occur in clusters, and which may have thickened, hyaline walls (Fig. 87 and Plate III). These tiny aneurysms, which doubtless, in large part represent the punctate hemorrhages of the clinical literature, seem to be most common

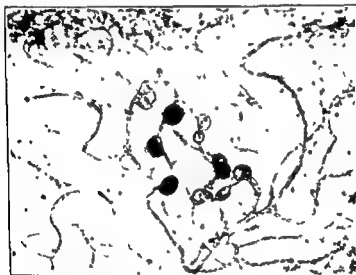


FIG. 87.—Diabetic retinopathy. Capillary microaneurysms demonstrated by the periodic acid-Schiff reagent in thin spread to whole mount of retina. (Dr. Jonas Friedenwald, courtesy of *Am. J. Ophthalm.* 42: 487, 1941.)

in diabetes, being found only occasionally in other conditions<sup>18,19</sup>—although Ashton,<sup>20a</sup> using injection methods, finds small peripheral aneurysms in about a third of nondiabetic eyes. The interesting question as to whether these lesions occur to any great extent in other tissues of the body has not been answered. A few have been described in the conjunctiva<sup>19,21</sup> and nail beds.<sup>19</sup>

Friedenwald<sup>19</sup> and also Ashton<sup>22</sup> cite the well-known association of this type of lesion with intercapillary glomerulosclerosis (see Chapter 12) and speculate as to whether there may be any pathogenetic relationship. Friedenwald<sup>19</sup> makes the interesting suggestion that both may represent a disturbance in the metabolism of mucopolysaccharides. This concept is enlarged upon in Chapter 28.

Ballantyne<sup>27</sup> also emphasizes the possible importance of venous stasis in the production of capillary dilatation and hemorrhage. Tortuosity and beading of the veins are fairly common and the occurrence of phlebosclerosis has been described particularly by Gibson and Smith<sup>28</sup>. They stress especially a hyaline thickening of the media of the retinal veins. Similar observations are reported by Agatston<sup>29</sup> and O'Brien and Allen<sup>30</sup>. The latter authors describe venous varices in the retina as being more common in diabetics than in patients suffering from arteriosclerosis and renal dis-



FIG. 88—Eccentrically thickened and flattened retinal vein.  $\times 500$

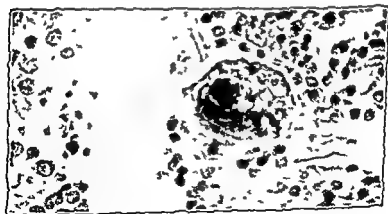


FIG. 89—Extreme proliferative reaction in wall of small retinal vessel (probably a vein).  $\times 500$

case (Figs 88-89). It should be emphasized here that arteriolar sclerosis is often seen particularly in the choroidal vessels but is similar to that found in nondiabetics.

The possible existence of an increased capillary fragility in diabetes as an explanation of the frequency of retinal hemorrhage has been suggested by Hanum & Wagener<sup>10</sup> and others, and has led to the treatment (without clear-cut results) of patients with vitamin C and various flavone derivatives, such as vitamin P (estrin), hesperidin and especially rutin. Rodriguez and Root<sup>11</sup> found increased capillary fragility in 40 per cent of 100 unselected diabetic patients; practically all of those having retinitis showed an in-

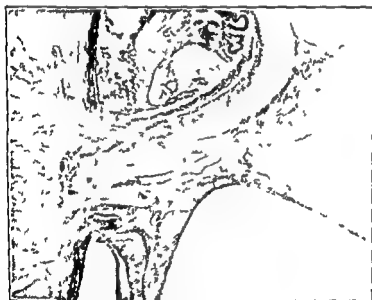


Fig. 90. Retinal capillary aneurysm in diabetes. The optic nerve is at the left. The hemorrhage is in the upper right quadrant. The optic nerve is at the bottom (above).

creased fragility. The fragility could be brought to normal by giving rutin

therapy with rutin.

The hard white exudates seen ophthalmoscopically in diabetes<sup>12,13</sup> are sometimes referred to simply as hyaline. Frieder<sup>14</sup> and Kovanagi<sup>15</sup> regard them as masses of inspissated protein, presumably coming from transudation of plasma through capillary walls. The ex-

regards them as characteristic of diabetes. Deposits of fat and cholesterol sometimes in phagocytes may also be seen.

Retinitis proliferans, which consists essentially in the growth of a connective tissue membrane from the retina into the vitreous, may be the unfortunate end result of diabetic retinopathy,<sup>26</sup> although it is not specific for diabetes. The pathogenesis is obscure, although it seems to be associated with the organization of repeated hemorrhages. Some authors emphasize the frequent association of this condition with the renal complications so often seen in diabetes of long duration. Allen<sup>27</sup> distinguishes two types of



Fig. 41.—Retinitis proliferans. Same as Figure 40 but (a) (b) (c) show detail of the proliferating vascular connective tissue membrane. Dark granular material in (a) (b) (c)  $\times 125$ .

retinitis proliferans. The first is due to inflammatory or traumatic alterations in the retinal vessels and is characterized by being located anywhere in the fundus and by the formation of a dense opaque connective tissue membrane rich in cells but containing few blood vessels. The second, which is the type found in diabetes, originates at or near the optic disk and is characterized by a profusion of newly formed blood vessels appearing in advance of any noticeable amount of connective tissue, and frequently showing a brush-like arrangement with coils or loops in the vitreous humor.

ing to the disk (Figs 90-91). Klien<sup>27</sup> considered the latter type as due to degenerative vascular disease leading to extensive anastomosis.

From the standpoint of the general pathologist, retinitis proliferans would seem to be most easily explained as equivalent to an organizing hematoma in other tissues. Its histologic pattern would then presumably be due to the characteristics of the vitreous, and its frequency in diabetics would be the result of the multiple retinal hemorrhages so common in these patients.

Another change not infrequently seen in the late stages of diabetic retinopathy is occlusion of the central retinal vein, either by thrombosis or intimal



Fig. 92.—Edematous intertumor diabetic. This may be seen in all stages of diabetic retinopathy.  $\times 200$ .

proliferation. This leads often to the formation of a peculiar connective tissue membrane on the anterior surface of the iris (Fig. 84).

In the late stages, and particularly in cases with advanced vascular disease of the kidneys, the lesions in the retina may be complicated by those of so-called "albuminuric retinitis," with marked arteriolar sclerosis in both the retina and choroid, and the deposition of fibrin nets (Fig. 92). These lesions are beautifully illustrated in color by Schieck.<sup>28</sup>

**Nerves**—The only change which appears to be distinctive for diabetes is the deposition of glycogen in the corneal and optic nerves, mentioned by Best<sup>2</sup> and fully described and illustrated by Hoffman.<sup>29</sup>

**Summary** In summary the following ocular changes may be said to be characteristic of though not necessarily specific for diabetes (1) vacuolation of and deposition of glycogen in the pigment epithelium of the iris (2) capillary hemorrhages and especially, microaneurysms in the retina (3) dilatation of retinal veins with phlebosclerosis (4) the deposition of dense protein rich transudates in the retina (5) retinitis proliferans (6) occlusion of the central retinal vein with formation of a vascular connective tissue membrane on the anterior surface of the iris

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Chapter 4

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## Chapter 14

### THE NERVOUS SYSTEM

ALTHOUGH diabetes mellitus is intimately connected with the nervous system (emotional glycosuria decreased carbohydrate tolerance during periods of mental anxiety) demonstrable changes in the nervous system are almost entirely due to complications of the disease rather than to diabetes itself.

The most striking changes are those secondary to vascular disease. Cerebral hemorrhage is much less frequent relative to other types of arteriosclerotic disorders in diabetics than in nondiabetics. The same holds true for cerebral infarction. Among 818 deaths of this series only 29 were due to cerebral arteriosclerotic changes. However De Long has reiterated the point of view that many of the neurologic manifestations of diabetes may be attributable to vascular disease.

Sections of the brain in Case No. 4106 dying as a result of massive intraventricular hemorrhage are shown in Figures 93 and 94. This case was a woman aged sixty-seven years whose diabetes had been present 21 years. The hemorrhage was secondary to rupture of the left middle cerebral artery. Of course changes of this type are in no sense peculiar to diabetes.

From the brains which we have personally examined and from the reports in the literature there are no changes distinctive of diabetes other than the abnormal glycogen deposits in certain cases of diabetic coma. We have not seen the droplets of glycogen described by Geipel<sup>1</sup> as occurring in the pyramidal cells of some cases of diabetic coma. Casanovi<sup>2</sup> has described the presence of droplets of glycogen in the perivascular lymph spaces of the brain in coma cases although these are frequently present in the infectious diseases as well. When the glycogen is present in large amounts droplets may be present in the glia cells. One of us (S. W.) has seen this type of glycogen deposition in 3 diabetic brains, 2 from coma cases. Other aspects of cerebral change will be discussed in Chapter 22.

The findings of Medwedeff and of Dillon, Riggs and Dyer in the brain in cases of coma are mentioned in Chapter 7. The specificity of such changes may be questioned.

A series of brains from the present series is now being studied by Dr. S. P. Hicks. His incomplete results suggest that there are no constant changes. Minor changes are of course hard to evaluate since diabetics are subject to such a variety of complicating diseases especially of the blood vessels. The findings of Vonderheide<sup>3</sup> and of Morgan et al.<sup>4</sup> of a reduced number of ganglion cells in the paraventricular regions have not been



confirmed, although Charr<sup>6</sup> has claimed to find degenerative changes in this part of the brain in a single case.

**Diabetic Neuropathy**—While neuritis particularly of the lower extremities is common in diabetes the underlying lesions are poorly understood. Paresthesia and anesthesia are not rare. Apparent trophic disturbances



FIG. 93.—Massive intraventricular hemorrhage from middle cerebral artery. No. 4006. Female, aged sixty-seven years. Duration of diabetes 21 years.



FIG. 94.—Hemorrhage in fourth ventricle from same case as above.

may occur.<sup>7</sup> Woltman and Wilder<sup>8</sup> have collected 35 cases from the literature and have reported 10 additional cases. In 3 of these the spinal cords were examined and showed only slight lesions, most easily explained on an arteriosclerotic basis and not similar to the degenerative changes of pernicious anemia. The posterior columns were the most involved. They

found in the peripheral nerves degeneration allegedly associated with marked sclerosis of the intraneural vessels and maintained that atherosclerosis is the most important factor in the lesions of nerves in diabetics. However their illustrations do not show the lesions usually regarded as atheromatous but rather what appears to be a mild concentric thickening of the walls of small vessels.

Other investigators<sup>9, 10, 10a</sup> have re-emphasized the rôle of sclerosis of the small vessels in diabetic neuritis. The evidence is not convincing however and it is not apparent that an adequate control series has been studied. Furthermore neuropathy of this type is not common in the advanced generalized arteriolar sclerosis of hypertensives, as one might expect if it were purely on a vascular basis.

Fraser<sup>11</sup> found a man aged thirty-six years whose diabetes had been present 12½ years with neuritic symptoms especially in the lower leg to have demyelination of the posterior tibial nerve.

As Woltman and Wilder<sup>8</sup> pointed out the pre-insulin diabetic neuritis was largely on a cachectic basis. While some<sup>12</sup> have suggested vitamin deficiency as a factor it is hard to think of a diet better provided with anti-neuritic vitamins than that of the typical diabetic. The rôle of vitamins is hard to assess.<sup>13, 14</sup> Rundles<sup>15, 16</sup> points out that vitamin therapy without control of the diabetes seems to have made some cases worse.

A group of nerves removed in this laboratory has been studied chemically.<sup>17</sup> The diminution of lipoids roughly paralleled the increase in arteriosclerosis. About one-half the cases had neuritic manifestations though

TABLE 33. AVERAGE VALUES WITH RESPECT TO ARTERIO-SCLEROSIS. DIABETIC COMPARED WITH NORMAL NERVES<sup>18</sup>

Arterio-sclerosis	No. of cases	Phospholipid per cent	Cholesterol per cent	Cerebroside per cent
1+ (slight)	1	3.85	1.15	1.60
2+	6	1.69	0.61	0.69
3+	12	1.67	0.60	0.77
4+ (advanced)	1	1.40	0.45	1.46
Normal average		4.40	1.97	1.73

frequent alimentary ailments of circulation might have played an important part in the symptoms. Efforts to correlate diabetic neuropathy with the chemical constituents of the blood<sup>19, 20</sup> have shown some elevation of the phospholipids in those patients showing neuropathy.

However the pathologic aspect of the problem has yet to be settled since clinical observation has shown diabetic neuritis to be independent of arteriosclerosis.<sup>21, 22</sup> Often occurring in young inadequately treated diabetics it may last for months only to disappear completely with adequate therapy. Thus far we have not had opportunity for histologic study of such a case. The pathology appears to be practically unknown.<sup>23</sup>

Sections of spinal cord have been studied in a number of our cases without definite abnormalities but we have not had material from a case of tabes diabeticus.

The prompt recovery in Major's<sup>23</sup> case suggests that the cord changes cannot always be severe.

Griggs and Olsen<sup>24</sup> described three types of cord involvement as occurring in diabetes: (1) degeneration of the motor cells of the brain stem and spinal cord; (2) degeneration of the intramedullary portion of the dorsal root fibers with secondary axon degeneration producing a lesion like tabes dorsalis; and (3) funicular necrosis of the posterior columns occurring especially in the cervical and upper dorsal segments of the cord. They report an example of the last type. The significance and pathogenesis of these lesions are obscure and their specificity may be questioned.

The rapidity of recovery in some cases of diabetic neuritis practically precludes any marked pathological change. This is evidenced by the case of Root<sup>3</sup>, a middle aged woman who had double wrist drop and double toe drop but made a good recovery in six weeks' time when she was given effective therapy for her diabetes.

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## Chapter 15

### THE PITUITARY

In the effort to find some explanation for the occurrence of diabetes in the absence of pancreatic pathology, clinical observation has led to the suggestion that pituitary disease is responsible for a certain number of cases. Marie<sup>1</sup> in describing acromegaly as a clinical entity, noted that diabetes occurred in 2 of his first 4 cases, and with the accumulation of case reports a definite relationship between these diseases became evident. The association of pituitary tumors and glycosuria had already been noted by Loeb.<sup>2</sup>

Cushing<sup>3</sup> in an admirable series of clinical observations and experiments produced strong evidence of the association of glycosuria with excess secretion of the pituitary, and conversely the association of an abnormally high carbohydrate tolerance with hyposecretion.

Presumptive evidence of the relationship of diabetes and the pituitary gland are the studies by White<sup>4</sup> and Joslin,<sup>5</sup> emphasizing the occurrence of diabetes among children who are taller than the ordinary, and who show other features suggesting pituitary abnormalities. Rosenbusch<sup>6</sup> however was not able to confirm this.

Table 34 gives some idea of the great frequency of diabetes or better glycosuria in acromegaly.<sup>\*</sup> The subject has been reviewed by Coggeshall and Root,<sup>7</sup> who also compared the organ weights of acromegalics and diabetics and found in general a predominance in favor of the former.

TABLE 34 FREQUENCY OF GLYCOURIA IN ACROMEGALY

Author	Cases of acromegaly	Number showing glycosuria	Per cent
Hunter and Osborn <sup>8</sup>	9	12	12
Hirschfeld <sup>9</sup>	130	14	11
Williams et al. <sup>10</sup>	21	6	29
Beckwith et al. <sup>11</sup>	176	71	40
Anders <sup>12</sup>	88	16	18
Rosenbusch et al. <sup>13</sup>	196	82	42
Cushing and Joslin <sup>14</sup>	100	27	27
Coggeshall and Root <sup>7</sup>	153	52	34

\* It must be emphasized that there is considerable overlapping of cases in this table. For instance, Coggeshall and Root include in their figures the 100 cases of Cushing and Joslin, which they reviewed again, finding the incidence of glycosuria higher than the original authors had thought.

One important difference between ordinary diabetes mellitus and that associated with hyperpituitarism is the tendency of the latter type to remain mild and even to regress. Two cases of Cushing (cited by Colwell) are known to have regressed after relief of pressure from the pituitary tumor.

The possibility that changes in the pituitary may hinder insulin formation by the pancreas is slight. Colwell<sup>15</sup> has collected 15 cases of acromegaly showing pancreatic changes which may be classified as follows: fibrosis, atrophy, hypertrophy, pancreatitis (hemorrhagic and suppurative), hyalinized islands, diminution of number of islands, and hyperplasia of island cells. Certainly no distinctive effect on the pancreas can be detected here. Root<sup>16</sup> has found no significant differences in the pancreatic weights of acromegalics with and without diabetes. It must be emphasized again that differential cell counts (see Chapter 3) have not been done in these cases.

Anterior lobe extract has been used by Young<sup>17</sup> to produce a permanent experimental diabetes in dogs (see Chapter 25). His results have been confirmed by others including Best.<sup>18</sup> Sections of a well preserved pancreas of one of Best's dogs examined by one of us (S. W.) showed marked hydropic change of the island cells. This is of special interest as assay of this pancreas showed it to contain no insulin.

Hydropic change (probably glycogen deposition—see Chapter 3) is characteristic of the early reversible stage of pituitary diabetes called idiopathic by Young. If injections are continued the pancreas may be permanently damaged and a permanent (metahypophyseal) diabetes ensues. This is discussed more fully in Chapter 25.

Doctor Young very kindly sent material from the pituitaries and pancreases of his dogs 28 and 41. One of us (S. W.) failed to find marked histological changes in either the pancreases or the pituitaries, although the insular epithelium showed apparent hydropic change. An incomplete cell count from the pituitary of dog 41 showed acidophils 43.2 per cent, basophils 5.9 per cent, and chromophobes 50.9 per cent. Some of the chromophobes were larger than usual. He reported in one of his dogs complete absence of normal island cells.<sup>19</sup>

Firmly established as is the influence of the anterior pituitary on carbohydrate metabolism, the role of this gland in human diabetes has yet to be clarified.

Attempts have been made to explain the ordinary type of diabetes on the basis of pituitary lesions. Fry<sup>20</sup> has described adenomatous proliferation of eosinophilic cells in the anterior lobe, areas of cellular degeneration and colloid invasion as characteristic of diabetes mellitus. Kraus<sup>21</sup> found similar lesions in the pituitary glands of 10 diabetics between fourteen and forty-one years of age, and in 2 of 12 cases over forty-one years of age. The increase was negative in only 1 case, however. He has found the pituitary in diabetes often small, even two thirds normal size.

Kraus<sup>22</sup> has described hydropic change of the basophilic cells in 2 diabetics whose pancreases also showed hydropic change. In the majority of the cases studied by him<sup>22</sup> there was a net reduction in the acidophilic elements when the size of the pituitary and the size and number of the acidophilic cells were considered although he also mentions adenoma-like masses of acidophils.

These findings have been accepted by many writers.<sup>23</sup> However it is interesting to note that when Kraus presented his findings at the meeting of the Deutsche Pathologische Gesellschaft in 1923<sup>24</sup> disagreement was voiced in the discussion that followed by at least two pathologists. The findings of Labbe and Petresco<sup>25</sup> tended to confirm those of Kraus.

Cunz<sup>2</sup> distinguished two kinds of chromophobe cells in the anterior pituitary: the stem cells and chief cells (Hauptzellen). The former type is the progenitor of all cells: chromophobe as well as chromophil and the latter type he apparently regarded as a differentiated but nongranulated cell. In 11 of 15 diabetics, most of them elderly, Cunz claimed to find an increase in these chief cells, usually associated with a diminution in acidophils. He likens the situation to a "shift to the left" or maturation arrest such as may occur in the bone marrow. He also recorded an increase in interstitial tissue in several cases.

If it be true that the growth hormone is the important one in experimental pituitary diabetes (see Chapters 25 and 26) and if it does play a role in some cases of human diabetes, one might expect an increased number of acidophils rather than the decrease noted by Kraus in most of his cases. In this connection Kraus<sup>22</sup> simply states: "Though the findings are not entirely uniform, there cannot be any doubt that while hyperfunction of the pancreas itself causes progressive changes in the hypophysis (such as hyperplasia and adenomas), a deficient function resulting in diabetes leads, particularly in experimental animals and in juvenile persons, to retrogressive lesions as described by Kraus." He also suggests that the discrepancy between his data and the negative findings recorded in earlier editions of this book may be due in part to the scarcity of juvenile patients in our series and in part to the effects of insulin treatment.

In a study of a series of 107 pituitary glands Parsons<sup>26</sup> included 7 cases of diabetes and one of hemochromatosis. No significant pathological change was found.

In 20 cases of diabetes Crooke<sup>20</sup> failed to find any histologic change in the basophilic cells.

Only one instance of moderate increase in number of basophils was found by Hawkins<sup>27</sup> among 6 cases of diabetes in which he did differential cell counts by the Rasmussen method.

We have been unable to confirm the findings of Kraus and of others who have reported characteristic findings in the pituitary in diabetes mellitus. As noted below, we have had hardly any acute untreated cases in young

patients and it is possible of course, that Kraus was fortunate in having a group of such cases before insulin came into general use.

Dr Louise Eisenhardt<sup>22</sup> has examined in serial section the pituitary glands of 18 of our cases of diabetes mellitus and random sections from 26 additional cases. She found no significant changes. Examination of additional random sections in our series has given no result at variance from hers. In her group of serially sectioned pituitaries the material was chosen to represent young diabetics whose disease was of long duration (2 cases) adults ranging from forty three to sixty nine years of age with diabetes of short duration and adults ranging from forty-eight to seventy four years of age with diabetes of long duration. Some of the patients had no insulin others had received slight amounts of insulin and others were under adequate control. One case died in coma. 12 showed mild diabetes and the remainder severe. The durations in these cases ranged from one month to eighteen and one-half years. Thus this group the glands of which were studied in serial section represents a very fair cross section of the diabetic population except for the omission of acute cases in the young. Autopsies have been very few on such cases in clinical colleagues. In the one case 48-91 studied through the courtesy of

who died acutely in coma the diabetes having been unrecognized prior to admission to the hospital we were unable to detect any definite changes in size of the gland or in distribution of cell types. There was no evidence of necrosis scarring or hyalinization.

In the 18 cases mentioned above the pancreases showed wide ranges of appearance. Some were completely negative others showed various changes such as diminution in number of islands, fibrosis or hyalinization of the islands, variation in size of islands and hemichromatosis. Fatty infiltration of the pancreas was noted in 5 cases.

These pituitary glands showed no constant significant change. The most constant findings were posterior lobe invasion by basophilic cells and slight to conspicuous vacuolization of these basophilic cells in the anterior lobe. The latter may be comparable to the change noted by Kraus in 2 of his cases associated with hydropic change of the islands of Langerhans. Since this vacuolization of the basophils occurred also in the control cases it is not regarded as a significant lesion. Basophils with two nuclei were common. An adenoma-like mass of basophils lay in the anterior lobe in Case 21757. The acidophils seemed to show no significant change in number except in one case that of a young male diabetic where the number was decreased and the cells were small in size. Some of the cells contained two nuclei. In 8 other cases multinucleated cells were present. Small vacuoles were frequently noted in the cytoplasm. In 2 cases the acidophils had homogeneous cytoplasm and one cell was found in mitosis.

Only one case therefore showed the diminution in acidophils that Kraus has stressed and none showed the adenomatous cell groups

that he mentioned. The chromophobes were noted as large in 4 instances but otherwise were not significantly altered except for the existence of vacuoles in occasional cases which gave some of them the appearance of foam cells. Nuclear pyknosis was common being present sometimes in all three cell types at times restricted to one or another. In 4 cases small foci of necrosis were encountered in the anterior lobe. In 3 cases there was a slight increase in interstitial tissue. Colloid in the anterior lobe was within normal limits. The colloid was particularly conspicuous in Case 20284 mild diabetes of four months duration occurring in a woman aged sixty years. The one case of hemochromatosis showed heavy pigment deposition throughout the anterior and posterior lobes. The pars intermedia showed colloid in all cases moderate in 9 and marked in 6. Cysts lined by cuboidal epithelium were present in 3 cases. Similarly no constant changes were found in the posterior lobe. Posterior lobe invasion by lymphohistiocytes was noted in 17 of the 18 cases and was marked in 6 and moderate in 5 cases.

We feel the conclusion is justified from these data that the pituitary gland presents no significant abnormality in diabetes mellitus with the possible exception of rare acute cases in the young.

There are two reports of apparent Housay phenomenon in man i.e. marked amelioration of a pre-existing diabetes seemingly due to destruction of the anterior pituitary by a coincident disease<sup>11,12</sup>

For a discussion of experimental pituitary diabetes see Chapter 25. For consideration of the possible etiological role of the pituitary in human diabetes see Chapter 26.

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## Chapter 16

### THE ADRENAL GLANDS

SINCE the demonstration that removal of the adrenals from a depancreatized animal would lead to amelioration of the diabetes<sup>1, 2</sup> and that administration of adrenal cortical extract to such an adrenalectomized depancreatized animal would again increase the severity of the diabetes<sup>4</sup> much additional evidence has been added especially by the work of Ingle<sup>5, 6</sup> on rats and that of Lorsham *et al.*<sup>7</sup> and particularly of Conn<sup>8, 9</sup> in producing a diabetic state experimentally in the human subject by administration of ACTH. Also of note is the work of Kobernick and Mori<sup>10</sup> in producing hydropic change (glycogen<sup>11</sup>) in the islets with cortisone. Further details of the experimental background for involvement of the adrenal glands in diabetes together with appropriate references may be found in Chapter 25.

Clinical evidence for the participation of the adrenal cortex in human diabetes has been provided in the first place by cases of Cushing's syndrome which is usually associated with either tumor or hyperplasia of the adrenal cortex and in which there is usually impairment of glucose tolerance and insensitivity to insulin as indicated by the glucose-insulin tolerance test.<sup>12, 13</sup>

Tumors of the adrenal cortex are commonly associated with diabetes usually of mild degree.<sup>14, 15</sup> Shepardson and Shapiro<sup>16</sup> studied 18 cases of adrenal tumor with diabetes and hirsutism (diabetes of bearded women); they noted no striking pancreatic changes in 8 autopsies other than one carcinoma and one case of hemorrhagic necrosis; however, specific granule stains were not used. In some cases cure of the diabetes has followed removal of adrenal cortical tumors.<sup>14, 17</sup> In such cases it is to be presumed that the diabetic state is accounted for by an overproduction of those cortical hormones which are chiefly concerned with carbohydrate metabolism, i.e. the 11 and 11-17 oxygenated steroids.<sup>18</sup>

Lukens *et al.*<sup>9</sup> in summarizing 55 cases of tumor or hyperplasia of the adrenal cortex found impaired carbohydrate tolerance in 49 per cent and well marked glycosuria in 45 per cent. Rossi and Blumenthal<sup>19</sup> reviewed 9800 autopsies with reference to the state of the adrenal cortex. Of the total 3 per cent had diabetes. There were 131 cortical adenomas and of these 21 or 16 per cent were diabetic. Of the 21, 16 also had hypertension.

Selye<sup>20, 21</sup> has extended his concept of stress to include episodes of hyperglycemia and ketosis following psychic or physical trauma and in fact includes diabetes among the diseases of adaptation. The implication is that oversecretion of adrenal cortical steroids in time of stress is the essen-

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show osteoporosis and an elevated excretion of 11-oxysteroids thus indicating a compensatory overactivity of the pancreas in order to prevent the hyperglycemia that would be expected. Finally carrying this reasoning over to diabetic patients they found in common with Miller and Mason<sup>22</sup> a somewhat decreased excretion of 17 ketosteroids and an essentially normal eosinophil response to ACTH. Thus they suggest that the activity of the pancreatic islet cells tends to vary directly with that of the adrenal cortex in man.

Also of interest in this connection is the work of Field and Marble<sup>23</sup> who studied the response to stress in 25 diabetics and 13 nondiabetics as measured by the eosinophil count on the second postoperative day following major surgery. Ten of the 25 diabetics showed an eosinophil count which was depressed less than 50 per cent of the preoperative level whereas all 13 controls had more than a 50 per cent fall in eosinophils. Also 5 of the diabetic patients showed less than a 50 per cent drop in eosinophils in four hours following a dose of 25 mg. of ACTH. (The same patients showed an adequate response to cortisone.) The conclusion was that some diabetics may have a diminished adrenal cortical reserve.

We have not observed any constant gross or microscopic changes in the adrenals in the present series. In a series of 50 cases the adrenal cortices have been measured in autopsy slides by the method described by one of us<sup>24</sup> and no significant change found. Virtanen<sup>25</sup> found no change in the weight of the adrenals in his cases.

**The Medulla.**—The adrenal medulla has never excited great interest as a possible causative factor in diabetes although the classical work of Cannon<sup>26</sup> established the hyperglycemia of sudden stress as due to an increased secretion of epinephrine. The reasons for this lack of interest have been apparently as Ricketts<sup>14</sup> states the transitory nature of the response to epinephrine, the failure to produce diabetes by long-continued injection of epinephrine, and the lack of effect of ablation of the adrenal medulla on the course of experimental diabetes. However as Ricketts<sup>14</sup> also points out human disease has excelled the laboratory in providing evidence that epinephrine may be diabetogenic. Since 1911 seven cases of pheochromocytoma have been reported in which the associated diabetes was cured or markedly ameliorated by removal of the neoplasm<sup>27-32</sup>. One of these cases had as much as 85 units of insulin daily. The actual mechanism of production of diabetes by these tumors is obscure and may perhaps be attributed to the action of epinephrine on the pituitary-adrenal axis<sup>33</sup> with secondary overproduction of cortical steroids. Thus the diabetes would be essentially of the adreno-cortical type.

The state of the pancreas in such cases of reversible diabetes due to adrenal tumors is so far as we are aware totally unknown except for the single case cited by Lukens<sup>32</sup> in which the  $\beta$  cells showed marked degranulation (early hydropic degeneration?) but had normal-appearing nuclei. It

trial factor (*et al.*) Hinkle *et al.*<sup>16</sup> and (Chapter 26). The enlargement and other changes in the adrenal cortex recently described in the course of alloxan diabetes<sup>24, 25</sup> are perhaps to be regarded as nonspecific effects of stress due either to toxic effects of the alloxan or to the diabetic state itself. In fact in any discussion of the role of the adrenals in human diabetes the possibility must be kept in mind that any changes encountered in the adrenals (such as those of Russi and Blumenthal<sup>10</sup> mentioned above) may possibly represent the nonspecific response of the organism to the stress of the disease state rather than having any primary etiological significance.

The rare concurrence of diabetes and Addison's disease<sup>16, 27</sup> is of interest. Usually the former has preceded the latter, and in some cases amelioration of the diabetes has been observed with the onset of adrenal cortical insufficiency, thus presumably reproducing the animal experiments mentioned above. Balfour and Sprague<sup>17</sup> describe two such cases. In one when the Addison's disease was treated only with desoxycorticosterone acetate the concentration of blood sugar decreased during fasting, the glycosuria and ketonuria were of only moderate intensity. However when the Addison's disease was treated with 11-dehydrocorticosterone (compound A) or 17-hydroxydehydrocorticosterone (compound L) the diabetes was greatly intensified and severe ketosis developed. Another interesting human experiment was performed by Green *et al.*<sup>28</sup> who removed in two stages not less than 98 per cent. of all adrenal tissue in a twenty-nine-year-old diabetic with severe hypertension. The patient had been diabetic since the age of six. Before operation the daily insulin requirement was 70 to 85 units and the blood pressure was 200/140. Six weeks after operation when the patient was being maintained on 15 cc. of cortical extract daily the insulin requirement was 25 units and the blood pressure was normal.

Hinerman<sup>29</sup> has made the interesting observation that the islands of Langerhans are uniformly hyperplastic in cases of Addison's disease and that while all cell types appear increased the  $\alpha$  cells show the greatest increase. Hinerman explains this hyperplasia as due to the removal of an inhibitory influence normally exerted in the presence of adrenal cortical hormones. We have been able to confirm his findings in a few cases but feel that the explanation is by no means clear and may be a nonspecific effect such as the  $\alpha$  cell hyperplasia which Ferner<sup>30</sup> obtained by repeated injections of insulin in the guinea pig.

Recently evidence has been brought forward that adrenal cortical function may actually be diminished in diabetes. Wilson *et al.*<sup>31</sup> have demonstrated that patients with Addison's disease respond with marked diabetic symptoms to a dose of cortisone which would have minimal effects in a normal person. They suggest that the Addisonian patient is habituated to a relative islet cell insufficiency as a compensatory protection against hypoglycemia. They also point out that by no means all patients with adrenocortical hyperfunction have manifest diabetes although they may

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is obvious that pancreatic biopsies adequately fixed and stained in cases of this type would be of extraordinary interest.

In the present series we have encountered 3 cases of pheochromocytoma. Unfortunately all were discovered unexpectedly at autopsy & it is not known whether the diabetes could have been reversed by extirpating the tumors. All were males around sixty years of age and all had had diabetes for at least six years. In no case was the presence of the tumor suspected clinically. Two died in postoperative shock, probably because of the presence of the tumor, the operation having been done for an unrelated condition. One was an incidental finding at autopsy. No significant changes were found in the pancreas of any of the three.

Sivers *et al*<sup>18</sup> have made the interesting observation that removal of the adrenal medullas from a strain of rats with hereditary diabetes resulted in a normal tolerance to injected glucose while administration of epinephrine to the demedullated animals would again give a diabetic type of glucose tolerance curve.

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sugar free. She died of nephritis three years later and at autopsy the thyroid showed a marked strumitis with almost complete destruction of the alveoli. The head of the pancreas was markedly fibrosed in the body of the pancreas many of the islands were hypertrophied some reaching three or four times the normal size.

Another instructive case is cited by Wilder.<sup>6</sup> This was a boy who developed diabetes when fifteen months of age. For six months he was kept on a fairly rigid diet and then this was somewhat relaxed without any evidence of glycosuria. A diagnosis of hypothyroidism was made when he was three years of age and with the institution of thyroid feeding intense glycosuria and acidosis developed. The thyroid administration was then discontinued and the tolerance increased. When seen by Wilder he was seven years of age and showed typical juvenile myxedema. Through administration of thyroid the metabolic rate was gradually raised to  $\sim 10$  and at that point sugar appeared in the urine. As much as 20 grains per day were excreted. Although the child gained in weight and strength sugar was present in the urine on a diet with a glucose equivalent of 76 grains and 10 units of insulin daily. Balfour and Sprague<sup>7</sup> give several case histories illustrating the effect of hyper- and hypothyroidism on diabetes including one young man who underwent a total thyroidectomy in order

to become more tractable (This result was indeed accompanied by myxedema).

TABLE 35. DIABETES ASSOCIATED WITH HYPERTHYROIDISM

No.	Age yrs.	Sex	Duration of diabetes yrs.	% c	Islands	Glucose	Character of lesion
4249	10	♀	0.2+	10 gm	Negative	Negative	Coma
12782	74	♂	0.0	10 to 15 gm	Trace	Marked glycosuria	Hypertrophic thyroiditis
11412	12	♀	0.5	70 gm	Negative	Negative	Rhomboid carotiditis
1326	21	♀	2.5	Negative	Negative	Slight glycosuria	Coma
11241	161	♂	1.0	1 gm	Negative	Moderate glycosuria	Toxic thyroiditis
5665	67	♀	1.5	Large	Negative	Glucose in urine	Glucose in urine
71006	61	♂	11	100 gm	Negative	Marked glycosuria	Diabetes mellitus (pre-diabetic)
51117	42	♀	1.5	60 gm	Slight glycosuria	Moderate glycosuria	Diabetes mellitus

11. A case of myxedema and diabetes. This man



## Chapter 17

### OTHER ENDOCRINE GLANDS

#### THYROID

SINCE the presence of hyperglycemia and glycosuria in cases of hyperthyroidism is not rare there is naturally a tendency to connect the disease with diabetes. John<sup>1</sup> pointed out that there is no definite relationship between the severity of hyperthyroidism or the height of the basal metabolism and the degree of hyperglycemia. It is natural to assume therefore that this is not due to the action of the thyroid itself but is due to a deficiency in insulin formation which is brought to light by the strain of hyperthyroidism.

This conclusion seems to be supported by the experimental production of diabetes by thyroid extract in animals already partially pancreatectomized (see Chapter 2).

Holst<sup>2</sup> found in 10 autopsied cases of diabetes associated with hyperthyroidism that there was no change in the pancreas in 4 in 6 cases; however the pancreas was small and in 4 of these the number of the islands of Langerhans was diminished. It is important in this connection to keep in mind the great difficulties facing any estimate of the number of islands present in the human pancreas and the wide range in number of islands known to be present normally<sup>3</sup> (see Chapter 3).

Various degenerative changes in the islands particularly necrosis or round-cell infiltration have been reported. It is possible to see how hypodrophic change of the insular epithelium might be present through long continued functional strain and possibly fibrosis may eventually develop in the islands.

However other changes are very difficult to associate. We have had an opportunity to study the pancreas of several cases of severe and long continued hyperthyroidism when the tissue was obtained very fresh. In the pancreas of these cases there was no change demonstrable. Garrod<sup>4</sup> however mentioned the occurrence of atrophic lesions of the pancreas in certain cases of exophthalmic goiter.

Some evidence has been produced for the occurrence of hypertrophy of the islands in cases of hypothyroidism or after thyroidectomy. One of the most interesting clinical evidences of this relationship is the one cited by Rohdenburg<sup>5</sup> in which a woman aged thirty years was found to be glycosuric and in spite of treatment could not be made sugar free. When she was forty years of age the menopause began and after this she became

In the female varying degrees of atrophy of the ovaries and uterus may occur largely proportional to the duration and severity of the diabetes. Most of the reports are in the older literature of the pre-insulin era. In two-thirds of Kraus' cases the ovaries were small, half or less the size of those of normal women. The development of follicles may be interfered with.<sup>12</sup> This may be a direct effect of the disease on the ovaries or it may be exerted through the pituitary. The former is more likely. The alterations are not specific for diabetes.

Kraus<sup>13</sup> ascribed the rarity of pregnancy in diabetic women first to the regressive ovarian changes leading to amenorrhea and sterility and second to the hindrance to conception offered by the catarrh of vulva and vagina so often present. In contrast with the past rarity of pregnancy in diabetes a large series of successful pregnancies has been reported by White.<sup>14</sup>

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tion. Post-mortem examination showed chronic thyroiditis, coronary sclerosis and an 80-gram pancreas with some large islands and some showing moderate hyalinization.

In the present series we have been able to study 8 autopsied cases of diabetes associated with hyperthyroidism. As can be seen from Table 3, the ages range from twenty-nine to sixty-seven years.

There is absolutely no characteristic picture in the pancreas. In fact the islands were negative in all except 2 cases where their number was apparently somewhat decreased and they showed varying degrees of fibrosis. The cause of death in one of the cases was carcinoma of the pancreas but many of the islands persisted uninjured in the midst of the carcinomatous tissue.

Total ablation of the normal thyroid has not been worth while as a means of controlling diabetes although sugar tolerance does increase.<sup>7,8</sup>

Hyperthyroidism even of long standing produces no lesion in the pancreas and conversely no characteristic lesion of the thyroid is found in diabetes. (We have not been convinced that the changes mentioned by Labbe and Petresco<sup>9</sup> are significant.) Changes in the Golgi apparatus and mitochondria of the thyroid possibly indicating hypofunction have been described in alloxan diabetes in the rat.<sup>10</sup>

### PARATHYROID

No consistent abnormality of the parathyroid glands has been found in these diabetic patients.

### PINEAL GLAND

No abnormality of the pineal gland has been encountered.

### THYMUS

In this series no thymic abnormality has been noted. Major has encountered 2 cases of apparent thymic enlargement. One of these was a girl aged eleven years dying in coma.<sup>11</sup> The thymus weighed 50 grams, the lymph nodes were enlarged and the pancreatic islands were few.

### GONADS

The sterility formerly noted in male diabetics is in all probability secondary to the malnutrition and frequent toxemias and attacks of acidosis of the poorly treated or untreated diabetic. We have noted no change beyond cessation of spermatogenesis and some increase in thickness of the basement membrane of the tubules. The interstitial cells are unaffected. In the adequately treated diabetic patient there is no apparent alteration from the usual picture.

islands present in different portions by the rough comparison of those countered in usual routine microscopic sections. On this basis 12 cases

relative insufficiency of insular tissue becomes actual as the slight factor of safety is reduced by injury from infection and other causes.

TABLE 16.—FATAL CASES OF DIABETES UNDER FIFTEEN YEARS OF AGE AT ONSET

Part A.—Deaths Prior to January 1, 1931

Case No.	Sex	Age at death yrs.	Duration yrs.	Cause of death	Islands of Langerhans	Interarteriosclerosis
78	♀	1 5	3 4 months	Coma		—
79	♂	2	1 mo.	Coma	Lymphocytic infiltration and hydropic change	—
865	♂	2 2	17 days	Coma		—
22-3	♂	2 9	"	Coma bronchopneumonia	Hydropic change pyknotic nuclei	—
2949	♂	1 8	1 1 yrs.	Coma otitis media	Lymphocytic infiltration	—
27 100	♂	4	2 wks.	Coma	Lymphocytic infiltration II+	—
27	♀	5 8	1 9 yrs.	Coma bronchopneumonia	Few	—
11 23	♀	6	6 wks.	Coma		—
11 23	♂	6	1 1 yrs.	Accident fracture of skull	Lymphocytic infiltration (colony type)	—
127 127	♀	1	8 wks.	Coma	Lymphocytic infiltration and hydropic change	—
343	♀	1	"	Septicemia		—
127 127	♀	12	5 days	Coma	Lymphocytic infiltration	—
127 127	♀	12	4 yrs.	Coma severe ill		+
21 101	♂	10	1 8 yrs.	Coma acute pericarditis		+
115 68	♂	11	11 months	Coma	Few hydropic change	—
122 71	♂	14	1 wk.	Coma	Slight fibrosis	+
1904	♂	15	1 6 yrs.	Coma	Lymphocytic infiltration	—
12 141	♀	15	1 mo.	Coma	Few	—
125	♀	15	8 months	Coma		—
121 10	♂	15 1	8	Coma	Few	—
119 8	♂	16	5 5 yrs.	Coma	Slight fibrosis	+
115 102	♀	16	2 1/2	Coma	Slight fibrosis and hydropic change	+

Part B.—Deaths from January 1, 1931 to September 1, 1935

Case No.	Sex	Age at death yrs.	Duration yrs.	Cause of death	Islands of Langerhans	Interarteriosclerosis
124 70	♂	8	4 3	Insulin reaction		III
28346	♂	10	12	Septicemia		+
10370	♂	14	8	Pulmonary tuberculosis	Few &+	+
34637	♂	14	"	Bronchopneumonia		—
325 78	♂	16	4 9	Insulin reaction	Few	+
10364	♀	17	2	Acute glomerular nephritis	II+	+
2160	♀	23	11	Menstrual coma		+

II = information missing

## Chapter 18

### THE PATHOLOGY OF DIABETES IN CHILDREN

In children alone may we hope to find pure diabetic lesions uncomplicated by any of the degenerative processes of age. Any changes which we find in the pancreas in children it is fairly safe to say are definitely associated with the diabetic process. For this reason it is discouraging at least from the standpoint of pathology that many of the pancreases in diabetic children fail to show any definite pathological change.

Of the 22 fatal cases up to 1930 reported in Table 36 but 4 had been treated with insulin for any length of time. A few of the others received insulin when already in terminal coma; they were first seen by a physician. Of the 4 cases treated for any length of time with insulin one was killed by an automobile, 2 were treated in the dawn of insulin therapy, and one a negro girl was treated for six months before death from coma precipitated by a severe sore throat, the duration of the diabetes being four years so far as known.

The cause of death in the diabetes of childhood in this first series was coma. In 5 cases coma was accompanied by infection sufficiently severe to be recognized at post mortem.

In the second series, largely drawn from insulin treated cases, infection is the important factor.

In the third series, while infection is still important, the longer period of survival causes cardiac and renal disease, especially the latter, to emerge as the major factors (*see also* Chapters 12 and 19).

It is noteworthy that among the 25 fatal cases since January 1, 1930, only two have died in coma. The increasing age at death of these childhood cases is also of interest in relation to the effectiveness of modern treatment.

While the pancreas is noted as small in many cases, this observation is uncertain. The size of the pancreas is far from constant even in normal persons, and the temptation is to regard as significant an organ slightly smaller than normal, paying no attention to those in the series that may slightly exceed normal.

Terbruggen<sup>1</sup> however was impressed by the small size of the pancreas in his juvenile diabetic group.

Quantitative studies of the amount of insular material present in the human pancreas are not yet feasible as routine procedures. The difficulties in the way of enumerating the islands of the pancreas are tremendous (*see* Chapter 3) and in none of this series has it been attempted. However from a number of sections it is possible to gain some idea of the various number

Perhaps the one most characteristic lesion of the islands in diabetic children is lymphocytic infiltration as already emphasized by one of us (S.W.). This lymphocytic infiltration present in 6 cases rarely occurs in older individuals. It strongly suggests the action of some toxic substance or possibly a mild reaction to the necrosis of island cells produced through functional overstrain. The short duration of these cases is very suggestive in so being six weeks or less and in the one case of longer duration being only nineteen months.

Reports of 5 autopsied cases of infants dying of diabetes up to one year of age were summarized by Lawrence and Mettence. Four of these showed lymphocytic infiltration of the islands. Two cases were apparently secondary to a generalized cirrhotic or inflammatory process in the pancreas but lymphocytic infiltration is obviously not a primary lesion of the islands but rather a response to pre-existing injury. Von Mevemburg calls it an "ulcer" and suggests that it may be due to functional overstrain (Leber listing).

Hydropic change was positively diagnosed in 7 cases and suspected in two cases of the present series. Hyalinization was encountered four times and fibrosis of the islands eight times. The islands seemed entirely normal from the qualitative standpoint in 13 cases and normal both in number and histology in 25. However the newer special stains (see Chapter I and Appendix V) were not used.

A fairly typical case is M.D. a boy aged fifteen years whose diabetes had begun 1½ years before death in coma. This was a pre-insulin case. The pancreas was small. The islands showed marked lymphocytic infiltration but their number was normal. There was no involvement of the acinar tissue. The heart was negative. There was marked glycogenic infiltration of the epithelium of Henle's loops and also of the liver-cell nuclei.

(Case H. 621) a boy aged fifteen years died in coma following a vomit throat 1½ years after the onset of diabetes which had been treated osteopathically. This case is not included in the table as death occurred shortly after apparent decrease in islands with some hydropic change. His stomach and intestines showed typical histological changes. Atheroma of the arteries was present. The liver weighed 1750 grams, showed much glycogen in the liver-cell nuclei and traces in the cytoplasm. Fat was present in moderate amount.

In this series the occurrence of large livers was not striking. In his study hepatomegaly in 5 per cent of diabetic children and Marble *et al.* have discussed in detail hepatomegaly as seen in (a) diabetic children. Atrophies of the pancreas accompanied by pituitary changes have been reported by Kraus in a diabetic boy aged fourteen years and a girl aged seventeen years.

The fact that hyaline degeneration, fibrosis and other irreversible tissue

Part I — Deaths from September 1 1937 to September 7 1948

Case No.	Sex	Age at death yrs	Duration yrs	Cause of death	Islands of Langerhans
44475	♂	11 mo	di conv 14 days	Undetermined	Lymphocytic infiltration Iyknotic nuclei H++ few
53419	♂	7	2 5	Undetermined	Hydropic change
108671	♀	14	10	Acidosis and coma	
30820	♂	15	1 5	Coma	* hydropic change diminished in number
63523	♂	16	5 5	Meningitis	I few
88704	♀	22	15	Renal insufficiency	S+++
43829	♀	23	17	Pulmonary tuberculosis	H+ I few small
80541	♂	25	15	Operative shock (pyelonephritis)	I few small
66101	♀	25	14	Renal insufficiency	I few small
80140	♂	27	14 5	Renal insufficiency resulting from pyelonephritis	Hydropic change I few small
77407	♀	29	16 4	Undetermined	
74831	♀	30	21	Undetermined	
80901	♂	30	18	Cardiac infarct	Abundant
104277	♀	30	23	Renal insufficiency	I few small
60891	♂	32	24	Myocardial infarction	Hydropic change 8-diminished in number
78704	♂	32	20	Pulmonary edema	S+ diminished in number
05181	♂	33	23	Cerebral embolism (cardiac)	
00127	♀	33	18	Renal insufficiency	.

Moore's case is of great interest. A thirteen year-old girl, with diabetes was of six years duration with but little effort at control,

In a case of a young man made available by Dr. L. D. W. LUND only were definite islands and even scars suggesting islands absent, but of the arterioles showed an extraordinary degree of hyaline thickening. Special stains were unsatisfactory, but suggested the presence of some cells. Diabetes had been present for sixteen years and coma had occurred five times. He died at the age of twenty-nine years from acute glomerulonephritis and bronchopneumonia. The liver was very large 3910 g. with a total fatty acid content of 3.23 per cent, within normal limit. Assay of the pancreas by Dr. G. T. Evans showed none present though the same method had demonstrated insulin in the pancreas of cases of diabetes.

Radiographic study of the bones of diabetic children has revealed multiple transverse lines of arrested growth in the long bones in some, probably associated with poor control of the disease."

While diabetes in children is usually severe, it becomes less severe with increasing duration of the disease, and there was one case in this series of which the duration was twenty nine years without the aid of insulin.

Allan and Wilder<sup>12</sup> in a study of 17 cases of diabetes in children which resulted fatally emphasize the potential severity of juvenile diabetes. Two deaths were due to intercurrent disease and one was moribund when treatment was started—the others were due to inadequate treatment. Unfortunately no autopsies were obtained.

White's<sup>13</sup> careful studies of diabetic children have done much to advance our knowledge in this field.

Priesel and Wigner<sup>14</sup> suggested juvenile diabetes to be a form of congenital insulin insufficiency associated with such degenerative stigmata as ab-  
sent ulnar dwarfism and bony malformations.

Ierner<sup>15</sup> has also put forth the concept of a congenital insular deficiency or rather inferiority ( *Minderwertigkeit* ) of the cells of the islands which prevents them from ripening in the normal manner.

The problem presented by infants of diabetic mothers will be discussed in Chapter 23.

White accepts two definite cases of congenital diabetes:

That congenital anomalies<sup>16</sup> may be important in rare instances is suggested by the case of Ghon and Roman.<sup>17</sup> An idiot boy aged fourteen years developed diabetes and died. The only pancreatic tissue was a plaque of pancreas within the duodenal wall and bulging into the lumen.

Rosenbusch<sup>18</sup> gives a broad discussion of childhood diabetes from a number of views including the psychiatric. He discusses a possible relationship between juvenile diabetes and von Nothmann's disease (glycogen storage disease) suggesting that both may represent disturbances of the autonomic nervous system, i.e. the hypophyseodiencephalic mechanism, the one tending to glycogenolysis, the other to glycogenesis. He seems to think that if one conceives of diabetes mellitus as a dysfunction rather than a hyperfunction of this mechanism, one may correlate with Kraus' findings of small pituitaries and diminished acidophils in cases of diabetes (see Chapter 15). Rosenbusch did not find in his series the acromegaly attributes observed by White in some of her diabetic children.

In recent years as juvenile diabetes has survived ten to twenty five years with the disease it has become painfully apparent that a very high incidence of vascular disease must be expected in this group. Thus Dillger<sup>19</sup> found that within a period of twenty five years duration of diabetes not one of 200 regularly examined patients escaped retinal hemorrhage regardless of age of onset, severity of diabetes or type of treatment used. He also found that retinopathy usually presaged progressive vascular



degenerative and that 51 per cent of the patients exhibited hypertension and albuminuria at the time of the earliest retinal hemorrhage.

Mann, Gardner and Root<sup>1</sup> described a disturbingly high incidence of intercapillary glomerular sclerosis associated with a nephrotic syndrome in young diabetics of long duration. In a series of sixteen fatal cases of renal disease complicating diabetes the average age at onset of diabetes was twelve, the average duration of diabetes at first signs of renal disease was 12 years, the average duration of diabetes at death was nineteen years and the average age at death was thirty-two years.

White and Walton<sup>2</sup> reported evidence of vascular disease in 82 per cent of 200 diabetics surviving twenty years of the disease. Chute,<sup>3</sup> Adams,<sup>4</sup> and Rosenbush<sup>5</sup> have commented on the appallingly high incidence of vascular damage in juvenile diabetics between the fifteenth and twentieth years of the disease.

As may be seen by comparing Table 36 above with Table 37 in the next chapter and is emphasized elsewhere, there is a striking preponderance of renal and retinal lesions in vascular disease affecting chiefly the capillaries in those patients whose diabetes begins in childhood. In fact efforts directed toward preventing these degenerative complications constitute one of the major therapeutic problems in the field of diabetes today.<sup>6,7</sup>

Considerable variation in opinion exists as to the importance of strict control of the diabetes in preventing these vascular complications. On the one hand there are those who advocate a fairly rigid control with diet as well as insulin aiming at as close regulation of the disease as possible<sup>8-12</sup> believing that hyperglycemia is harmful.<sup>13</sup> Jackson and his group<sup>14</sup> have been especially emphatic in holding that good control of the young diabetic is both desirable and possible. Wilson, Root and Marble<sup>15</sup> reporting on a group of 247 patients whose diabetes began between the ages of eighteen months and thirty years and had been in existence from ten to thirty-four years, have brought forth evidence suggesting that poor control leads to a higher incidence of vascular calcification and retinal lesions than is found in patients under good or excellent control. Similar conclusions were reached by Root, Simlen and Zancs.<sup>16</sup> However, as noted elsewhere it is a depressing fact that Wilson *et al.*<sup>15</sup> were able to place only 7 of 247 patients in their excellent control group and 30 in the good control group. On the other hand is the school which holds that a liberal regimen with more or less constant hyperglycemia and, although but with a daily dose of insulin, is more beneficial from the standpoint of preventing the vascular and several other complications which predispose to the long-term complications of the disease in young diabetics. (See Chapter 37.)

diabetics (See Chapter 37.)

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## Chapter 19

# THE PATHOLOGY OF DIABETES IN CASES OF LONG DURATION

Of no little interest are those cases of diabetes which have successfully resisted the disease for a number of years. In this series of 809 cases of diabetes which came to post mortem examination 157 or 18 per cent were of fifteen years duration or longer in contrast to the 300 cases of the first edition in which only 23 or 8 per cent had lived fifteen years with the disease. Now furthermore there are 71 cases of twenty years or longer duration and 26 who passed the twenty five year mark before they died. There are two factors to be considered in these cases (1) The local duration of diabetes has given full range for the development of pathological lesions secondary to it and (2) there has been ample opportunity for the degenerative processes of age to complicate the diabetic portion of the picture.

The case of longest duration in this series is No 511 a male aged seventy years whose diabetes had developed 39 2 years before death. The immediate cause of death was an acute pleuritis accompanied by vague respiratory symptoms. He was well nourished and well developed. The pancreas weighed 50 grams. The islands were normal in number and showed moderate hyaline change and slight fibrosis. There was marked fatty infiltration of the acinar tissue and marked interlobular and interacinar fibrosis with scattered small retention cysts associated with the ducts. The heart weighed 500 grams the hypertrophy being chiefly of the left ventricle. There was a healed infarct of the left ventricle and marked myocardial fibrosis. The coronary arteries were tortuous showed numerous atheromatous plaques and were markedly calcified. The liver was essentially negative. There was a large cholesterol stone in the gall bladder. The kidneys were of moderate size and both showed a moderate degree of vascular scarring with some sclerosis of the large vessels. The aorta showed extreme atheromatous degeneration with marked calcification. There was no gangrene. In other words in this case the diabetic lesions seen were relatively inconspicuous as compared with the lesions which we may be justified in considering as secondary to arteriosclerosis.

Another case of 33 7 years duration a woman aged eighty four years died from coronary thrombosis. She weighed 138 pounds after one leg had been amputated at mid thigh. The pancreas weighed only 40 grams. The islands were normal in number and showed a moderate degree of fibrosis. There was a moderate degree of interacinar fibrosis as well. The

heart was moderately enlarged weighing 440 grams and showed marked myocardial fibrosis as well as a healed infarct. No fresh infarct had developed from the thrombosis owing to an insufficient lapse of time. The coronaries were markedly atheromatous and calcified and the left was thrombosed. The liver weighing 1100 grams was negative. The kidneys were slightly contracted and showed a moderate degree of vascular scarring. The aorta showed marked atheromatous change with calcification. The left leg had been amputated for gangrene. There were many gall stones of the cholesterol type accompanied by a moderate degree of chronic cholecystitis. The eyes showed bilateral cataracts. In this case just as in the other while the pancreas showed clear-cut pathology a considerable number of apparently normal islands were present.

It speaks well for the treatment of the present day that several of these cases have outlived their normal expectancies of life from the age at which they first developed diabetes.

However by and large diabetics do not have a normal life expectancy. Millard and Root<sup>1</sup> present an analysis of 110 cases concluding that the average life expectancy fulfilled by these patients was only 44 per cent of normal.

The causes of death in these cases differ strikingly from those of Nannini<sup>2</sup> in pre-insulin days over half of whose cases died of coma or tuberculosis. Coma as a cause of death was noted in only 1 of the present group of 157 cases and 1 of these occurred in the days before insulin. In 1 of these cases the islands were noted as few. The occurrence of definite pancreatic lesions in many of these mild cases of long duration and the relative absence of these lesions in the severe cases of short duration in the children is just the reverse of what might be expected in attempting to correlate pathology and symptomatology. A glance at Table 17 shows that one-half of the cases showed some degree of hyalinization or fibrosis of the islands. Three showed evidences of hypertrophy of the islands. 22 showed few islands and 3 showed probable hydropic change.

Over one-fourth the cases showed no demonstrable pancreatic pathology with the usual routine stains.

Most striking of all is the extraordinary incidence of atherosclerosis especially of the coronary arteries as a cause of death. The study of Cline<sup>3</sup> as cited in Chapter 11 showed only about 3 per cent of deaths in a large autopsy series to be directly attributable to coronary sclerosis. In the table above fully 43 per cent of deaths are directly ascribable to coronary atherosclerosis and most of the others of course had atherosclerosis though it was not listed as the chief cause of death. Vascular disease and infection together account for 85 per cent of the deaths emphasizing in a most striking manner the two major problems in the management of diabetes.

TABLE 37 CASES OF DIABETES > FIFTEEN YEARS DURATION OR MORE (17 CASES)

No	Age	Duration	Cause of death	Islands	As to arterio- sclerosis	Compensatory	Cardiac	Compensatory
19	57	15 0	M		+++	-		
371	54	1 0	M		+	-		
1901	61	1 0	M		+	-		
209	78	1 0	Cardiac infarct	H+++	+	-		
1879	67	15 0	Cerebellar hemorrhage	H+++	+++	-	1 fresh	
1991	50	15 0	Chronic bronchopneumonia	Few	++	-		
818	43	15 0	Cerebral thrombosis	+++	+++	-		
9413	64	1 0	Infarct of brain	H+++	+++	-		
4098	5	15 0	Myocardial infarct	H+++	+++	-		
4344	4	1 0	Myocardial infarct	H+++	+++	-		
464	68	15 0	Renal infarct	H+	+++	-	1 fresh	
49378	60	1 0	Renal infarct	H++	++	-		
3749	71	15 0	Coronary atherosclerosis	H+	++	-		
951	6	1 0	Myocardial infarct	H++	+++	-	1 fresh	
753	7	1 0	Myocardial infarct		+++	-		
79317	30	15 0	Myocardial infarct		+++	-	1 fresh	
8049	1 0	1 0	Myocardial infarct		+++	-		
8300	63	1 0	Myocardial infarct	1 fresh	+	-		
83783	48	15 0	Myocardial infarct	H+++	+++	+		
87877	48	15 0	Myocardial infarct	H+++	+++	+		
88704	20	15 0	Renal infarct	H+++	+++	+		
89600	60	1 0	Myocardial infarct	H+++	+++	+		
91970	5	15 0	Myocardial infarct	H+	+++	-	1 fresh	
91791	74	1 0	Myocardial infarct		+++	-	Healed	
978	43	1	Myocardial infarct		+++	-	1 fresh	
89	64	1 3	Myocardial infarct	+++	+++	+	1 fresh	
97740	64	15 3	Myocardial infarct	+++	+++	+	1 fresh	
D427-4	76	1 5	Myocardial infarct	+++	+++	+	1 fresh	
7073	0	1 5	Myocardial infarct	+++	+++	+	1 fresh	
32078	66	15 8	Myocardial infarct	+++	+++	+	1 fresh	
87404	67	1 8	Myocardial infarct	+++	+++	+	1 fresh	
18	60	16 8	Myocardial infarct	+++	+++	+	1 fresh	
31661	61	10 0	Myocardial infarct	+++	+++	+	1 fresh	
3194	60	16 0	Myocardial infarct	+++	+++	+	1 fresh	

H = hyaline  
Post mortem examination limited

R = fibrous  
T = tumor

M = metastatic



TABLE 27 CASES OF DIABETES > FIFTEEN YEARS DURATION OR MORE (10 CASES)

No.	Age	Duration	Cause of death	Islands	Is atherosclerosis	Cardiomegaly	Cardiac infarct	Calcification
19	47	10 0	M		+++	-		
3361	44	10 0	M		M	-		
19021	61	10 0	Sepsis	H+++	+	+		
27093	78	15 0	Cardiac infarct	H+++	+++	-	Pericard	
17879	69	10 0	Cerebellar hemiparesis	H+ I em	++	-		+
17069	50	15 0	Coma from hypoparathyroidism	Fem	+	-		+
8178	43	10 0	Sepsis		+++	-		
9413	64	10 0	Cerebral thrombosis	H++	+++	-		
40 88	6	10 0	Intestinal embolism	H+++ G+ aneurysm	+++	+	Yes	
4344	74	10 0	Myocardial infarct		+++	-	Fresh healed	
4640	68	10 0	Sepsis	H+	++	-		
49578	60	10 0	Renal insufficiency	H++	++	-		+
3849	71	15 0	Coronary atherosclerosis	H+	+++	-	Yes	-
40 81	6	10 0	Myocardial infarct	H++	+++	-	Fresh healed	-
37 37	9	15 0	Angioma of the aorta		+++	-	Yes	-
69317	3	10 0	Leukemia	Leukemia	+	-	Healed	
80 49	2	10 0	Operative shock (peritonitis)	Fem small	+	-		
83 02	43	10 0	Intestinal embolism	H+++ aneurysm	+	-		+
83783	6	10 0	Undetermined		+++	+		-
87877	48	15 0	Sepsis	H+++ skin large	++	-		-
88704	22	15 0	Renal insufficiency	G+++	+++	+		-
8 800	69	10 0	Myocardial infarct		+++	-	Yes	-
91970	6	10 0	Coronary thrombosis	H+	+++	-	Healed	+
101 00	44	15 0	Myocardial infarct		+++	-	Healed	-
78	44		Infarct of the left ventricle		+++	-		-
84	46	10 3	Infarct of the left ventricle	H+++	+++	+	Healed	+
7740	79	10 3	Cerebral infarct		+		Healed	+
14274	6	15 0	Brain tumor	H+	+++	-		
2 033	70	15	Septicemia	H+++	+++	+	Pericard	-
32 78	60	15 8	Coronary atherosclerosis		+		Fresh	-
87404	63	15 8	Myocardial infarct		+++	-	Healed fresh	+
18	60	15 0	M		+	-	Fresh healed	+
31640	61	16 0	Myocardial infarct	H+++	++	-		+
3194	60	16 0	Cerebral arteriosclerosis	H+++ I em	+++	-		

H = Hospital

\* = Post mortem examination limited

G = Good

F = Fair

M = Information missing

TABLE 27 -- (Continued)

No.	Age	Duration	Cause of death	Islands	Lesions a. Coronary arteries	Gen gross	Cardiac infect	Gall stones
10,304	44	70 8	Myocard	None	+++	+		-
13707	74	20 3	Myocard	II++	+++	+		-
6778	67	20 7	Infarct of heart	II++ III++	+++		Fresh healed	
23442	51	21 0	Coronary throm bosis		++		Healed	
27249*	60	21 0	Cardiac failure		VI		VI	+
44796	60	21 0	Coronary athero- sclerosis	II+	+++		Fresh healed	
74431	30	21 0	Indeterminate		++			
107070	67	21 0	Cardiac failure (cause?)	Large	++			+
82,03	74	21 3	Myocardial in- farction	II+	++		Yes	
3772	63	21 1	Coronary artery disease		+			
1287	70	1 8	Coronary artery disease	II+ III++	+++			+
27077	71	21 8	Bronchopneumonia	II+++	+++			
26,47	50	22 0	Coronary throm- bosis	Few	+++		Fresh	
28048	60	72 0	Arteriosclerosis	II+++	+++			
8,448	67	27 0	Dilatative cardio- myopathy	Few	+			
91971	III	27 0	Pericarditis	Small	+++		Healed	
9707	50	22 0	Coronary artery disease	Few hydropericardium	+++		Healed	+
107776	73	0	Infarct of heart (cause?)		++			
21546	60	72 4	Coronary artery disease	II++	+++			+
60	72	22 2	Coronary artery disease	II drug change small	+++	+		
81512	73	72 4	Myocardial in- farction	None	+++	+	Fresh	+
101790	60	22 5	Myocardial in- farction		+++			
3564	68	71 0	Coronary artery disease	II+++	+			
80411	50	23 0	Myocardial in- farction	Adenoma	+++		Yes	
80014	84	23 0	Coronary artery disease		++			
79381	33	21 0	Coronary artery disease		+++	+	Yes	
104277	30	23 0	Pericarditis	Few small	+++		Healed	
10416*	71	71 0	Myocardial in- farction	II++	++			
1701	50	23 2	Coronary artery disease	II+++	++	+		
17012	64	24 0	Coronary artery disease	II+	++	+		
7007	77	74 0	Myocardial in- farction	++ Pericarditis	++		Fresh healed	
85073	71	20 0	Postoperative re- sult of aortic aneurysm		++			

If hydropericardium

+ Fibrous

30 infarct area present

\* Post-operative aneurysm on left ventricle

+ Scar



TABLE 37 — (Continued)

No	Age	Duration	Cause of death	Islands	Aorta arterio- sclerosis	Gen erous	Cardiac infarct	Gall stones
75794	68	18 0	Myocardial in farction		++	-	Free	+
70911	61	18 0	Myocardial in farction	H + few pyk notic nuclei	+++	-	Free	+
60901	30	18 0	Myocardial in farction	Many	+++	+	Free	-
93527	67	18 0	Undetermined		+++	-		-
86127	33	18 0	Renal insuff iciency		+++	-		-
26183	80	18 3	Coronary occlu sion		+++	-		+
27013	67	18 5	Coronary occlu sion	H+++ S+	+++	-	Free	
22075	57	18 6	Coronary insuff iciency	Large few S+	+++	-	Free	
20051	71	19 0	Sepsis	H+	+	-		
38787	71	19 0	Multiple ab scesses	S+ few	+	-		
50132	71	19 0	Coronary occlu sion	few	+++	-		
63825	74	19 0	Myocardial in farction		++	-	1 cm	-
71381	71	19 0	Coronary throm bosis	H+++	++	-	Free healed	-
73853	72	19 0	Sepsis		+++	+	1 cm Healed	+
22082	40	19 0	Undetermined probably cor onary occlu sion	S++	+++	-		-
100320	73	19 0	Nutacate myo cardium	H++ S++	+	-		-
84706	71	19 1	Rheumatic heart disease		+++	-		-
22573	19 2		Sepsis		-	-		-
24351	82	19 4	Undetermined		+++	+		+
17832	73	19 5	Rupture of heart	H+++ few	+++	-	Free with rupture	-
24861	19 7		Pulmonary em bolus		+++	-	Healed	-
28129*	64	20 0	Bronchopneu monia	M	M	-	M	+
28536*	71	20 0	Pneumia	H+++	M	-	M	-
15007	71	20 0	Acute pericard itis	H+++ S+	+++	-		+
62711	52	20 0	Myocardial in farction	H+	+++	-	Free healed	Organ removed
3330	71	20 0	Coronary occlu sion		++	-		-
5190	80	20 0	Hypostatic pneumonia and cachexia (cancer)		+++	-	? infarct	+
7014	70	20 0	Myocardial in farction		+++	+	Healed	+
73	71	20 0	Pulmonary em bolus		++	+		+
78	71	20 0	Pulmonary edema	4+ few	+	-		-
70	70	20 0	Coronary insuff iciency		+++	-	Healed	M
71	71	20 0	Pulmonary in sufficiency		+	-		-
71	71	20 0	Cardiac tam ponade		+++	-	Free rupture	Organ removed

H—hyaline

\*—Post mortem examination limited

S—fibrous  
f—fibrin

M—information missing

These findings correlate well with those of Millard and Root<sup>1</sup> who concluded that 85 or 77 per cent of 110 diabetic patients had evidence of atherosclerosis of clinical importance. Likewise Robbins and Tucker<sup>2</sup> found that in their series of 307 cases coronary occlusion, peripheral vascular disease, infections of the extremities and acute pyelonephritis were particular hazards for the diabetic patient.

The problem of the possible relationship between this extraordinary incidence of atherosclerosis and the high fat diets which many of these patients had during the early part of their disease is a vexing one, and one which will probably not be answered until serious work with a large number of cases treated on a low-fat regimen becomes available (see chapter 9).

It is interesting to compare the table above with the table in the preceding chapter (page 211). It becomes evident at once that as a cause of death renal disease is more important in the younger age group, coronary disease in the older. This situation can be interpreted in at least two ways. It could mean (1) simply that the people in the older group lived long enough to develop advanced atherosclerosis, or (2) that the diabetics of older group and hence tends to be older (perhaps also in kind?) from that of the type of vascular damage. It is conceivable of course that the older group would eventually have succumbed to the renal type of lesion if atherosclerosis had not been superimposed. These questions do not seem to be resolvable in the present state of our knowledge.

As mentioned in the preceding chapter the question of the relation of the degree of control of the diabetes to the incidence of vascular complications is a controversial one. Wilson, Root and Marble<sup>3</sup> in a series of 101 patients who had had diabetes more than twenty years found a distinctly higher incidence of vascular complication and of retinal lesions in those whose diabetes was under poor or fair control than in those under good to excellent control. However, out of their entire series of 247 patients only 7 were able to qualify as having been under excellent control. Doherty<sup>4</sup> on the other hand finds no relation between control of the diabetes and the incidence of vascular disease. (See also the recent summary by Jordan<sup>5</sup>.)

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TABLE 37—(Continued)

No.	Age	Duration	Cause of death	Islands	Porto arterio- sclerosis	Con- gest	Cardiac infarct	Gall- stones
2400	74	24 3	Cerebral softening	H+	+++	-		+
9974	78	2 0	Infarct of heart	H+++ S++	+++	-	Healed	-
U2313	77	2 0	Coronary thrombosis	Leu	++	+	Healed	-
M389	2	24 0	Myocardial infarction	H+++	M	-		-
1074	73	2 0	Coronary infarction (cancer)	H+++ M	+	-		-
10948	71	2 0	Infarct of heart	H+++ a few aneurysms	+++	-	Healed	-
100910	72	24 0	Pulmonary embolism		+++	-		-
7104	75	25 2	Streptococcal sepsis	H++	+++	+	Fresh	+
D42707	84	24 0	Cerebral hemorrhage	H++	+++	-	Fresh healed	-
3706	71	26 0	Septicemia	H++	+++	-		Osgood's removal
6177	73	26 0	Undertaken pericardial emphysema	H++ S+	+++	-	Healed	-
10640	77	26 0	Coronary infarction myocardial infarction	H+++ S+ Exemplary filtration	+++	+	Fresh	-
1003	63	9 1	Coronary infarction	H33 retrograde	+++	-	Healed	-
1115-102	38	24 0	Coronary infarction	Leu	M	M		M
9298	74	29 0	Coronary infarction	Leu	+++	-	Fresh	+
4090	70	29 4	Infarct of heart	Few H+	+++	+	Fresh with rupture	+
812	77	30 0	Bronchopneumonia	Few H+++ S+++	+++	-		-
23083	71	30 0	Myocardial infarction		++	+		-
97543	66	30 0	Coronary infarction	Small	+++	-		-
M890	64	30 2	Gastric cancer	H++	M	+	M	-
104079	61	32 0	Undetermined probably cerebrovascular (cancer)		+	-		+
50016	79	33 0	Coronary thrombosis	H+ S+	+++	-	Fresh	-
6365	84	33 7	Coronary thrombosis	S++	+++	+	Thrombus healed	+
3870	73	3 0	Coronary infarction	S+ Few	+++	+		-
3804	66	32 1	Pulmonary embolism	H33 retrograde	+++	-		-
10175	87	36 0	Coronary infarction	H+++ Thick of coronary artery few irregular aneurysms and shape	+++	-		+
all	70	39 11	Acute leukemia	H+++ S+	+++	-		+

H—by an anastomosis

S—Post-mortem examination limited

S—fibrous  
t—tubercles

M—informal on the neck

become necrotic, they are desquamated and pass out with the urine. One of the means of diagnosis of the disease is the finding of iron-containing epithelial cells in the urine.

The clinical aspects of the disease are summarized by Marble and Bailey,<sup>1</sup> who reported 30 proved cases with skin or liver biopsies. Of these 27 died, the average age at death being 57.8 years and the average duration of the diabetes 4.9 years. Many showed some resistance to insulin (see also Chapter 21). These authors emphasize the frequency of hypogonadism as part of the syndrome and describe methods of diagnosis. The recent popularity of needle biopsy of the liver has provided a new and useful

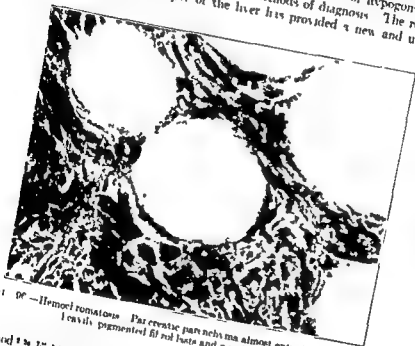


Fig. 96.—Hemochromatosis. Parenchyma almost entirely replaced by heavily pigmented fibrotic tissue and many ring cells.

method.<sup>2,3</sup> Fishback<sup>4</sup> has described a method for demonstrating iron in the skin of the living patient by injecting potassium ferricyanide and dilute hydrochloric acid directly into the skin.

The initial step in the genesis of hemochromatosis, the formation of the pigment, is as yet not clear. Nevertheless the effect of the pigment and the correlation of the lesion with the clinical course of the disease are very thoroughly known. In other words we have in cases of hemochromatosis with diabetic symptoms practically an experimental diabetic condition produced by the action on the pancreas of a known substance. Hemochromatosis without severe involvement of the pancreas does not cause diabetes mellitus. In cases where there is severe pancreatic involvement

except that the course is more rapid and that insulin resistance may be a prominent feature. Here then we have in ideal means of studying diabetes with a known etiology and a fairly rapid course.

Bronze diabetes was first described clearly by Hanot.<sup>3</sup> The bronzing of the skin is of course not a necessary concomitant of the diabetic syndrome for it largely depends on how much of the iron has been deposited in the skin. There is not only deposition of iron in the skin but there is also well an increase of the melanin in the skin causing still further pigmentation.<sup>26</sup>

Hanot<sup>3</sup> felt that the diabetes was the underlying factor in the condition. However, Opie<sup>2</sup> pointed out that the 'bronze diabetes' of Hanot is identical with the hemochromatosis of von Recklinghausen.<sup>4</sup> Two pigments are involved: the one in which iron can be demonstrated, hemosiderin, and the other in which iron cannot be demonstrated, hemofuscin. Mallory<sup>5</sup> believed that hemosiderin was the product of the intracellular digestion of hemofuscin and that both were derived from the blood pigment.

More recently there is a tendency to doubt that hemofuscin is derived from the breakdown of hemoglobin and to consider it as identical with the wear and tear pigment of the older pathologists and as possibly related to melanin.<sup>8</sup> It seems probable that several different pigments are described in the literature under the name of hemofuscin. As indicated by Sheldon<sup>9</sup> and the Gillmans,<sup>10</sup> staining methods may be important.

It is only in the most advanced cases, as a rule, that hemochromatosis produces diabetes. Usually death occurs from hepatic insufficiency before this event or the patient dies from some intercurrent infection. Of 10 cases of hemochromatosis autopsied in Mallory's laboratory none showed sugar in the urine although they were well developed cases of the disease. The frequent association of exposure to copper with these cases led Mallory to believe that it might be an etiological factor and animal feeding experiments enabled him to produce through the administration of small amounts of copper over a long period of time lesions very similar to those of hemochromatosis. Other workers, however, have not been able to confirm Mallory's observations and Sheldon<sup>9</sup> after a careful critical analysis concluded that there is insufficient evidence to incriminate copper.

At present the etiology must be still regarded as *sub judice*. There is a strong possibility that the disease is due to a defect in iron metabolism especially since iron retention has been demonstrated in some cases.<sup>11</sup> Sheldon<sup>9</sup> in an exhaustive survey of the literature and a closely reasoned analysis concluded after eliminating various other hypotheses that hemochromatosis should be considered as due to an "inborn error of metabolism" a theory given support by the occasional occurrence of the disease in siblings (see also Rogers<sup>27</sup>). According to this point of view the storage of iron

continues throughout the life of the individual not reaching a totipotency sufficient to produce symptoms until middle life thus explaining the well known age incidence. The marked predominance in males is not elucidated by this theory (unless it be assumed that the menstrual blood loss in the female prevents the disease from becoming manifest).

As noted by Warren and Drake<sup>11</sup> there has been in the past a tendency to reject the diagnosis of hemochromatosis if a known etiology as a frequentiation according to Sheldon<sup>12</sup> has been that idiopathic hemochromatosis showed a predominance of hemoviderosis in the liver skin and pancreas also in the epithelium of the gastrointestinal tract in the endocrine glands and choroid plexus while hemoviderosis as in hemohætic meningitis involved chiefly the reticulo-endothelial system.

However recent reports of apparently classical hemochromatosis developing in cases where multiple transfusions were given<sup>13</sup> have tended to broaden the general concept of the etiology and pathogenesis of the syndrome (as it should perhaps be called) of hemochromatosis. However even here the pathogenesis is not so clear as it might be since frequently evidence of hemohæsis is lacking and indeed the amount of iron found may be greater than the calculated amount provided by the transfusions.<sup>14</sup> It is not entirely clear in the present state of knowledge that a distinction can be made between exogenous hemochromatosis and the endogenous disease as it

Recently there has also been a tendency to consider dietary deficiencies as playing a major rôle in the development of the disease a tendency which has been accelerated by the work of the Gullmans<sup>15</sup> in South Africa. These authors after studying 400 liver biopsies in 120 cases of pellagra feel that there is quite with assurance that hemochromatosis can be regarded as one of the commonest sequelæ of pellagra.<sup>16</sup> They hold that the fundamental defect in hemochromatosis is a profound disturbance in intracellular metabolism induced by dietary imbalance. They believe that both hemochromatosis and hemofuscinosis have a common origin from mitochondrial defects suggested the names *cytosiderin* and *cytolipochromin* respectively to replace the old and misleading terms.<sup>17</sup> The work of these authors is apparently carefully done and instructive but it does not seem to justify their sweeping assumption that the disease in South African pellagrins is thereby settled. The fact that the disease in South African pellagrins is different from classical idiopathic hemochromatosis. Also hemoviderosis does not seem to be characteristic of pellagra as seen in this country.<sup>18</sup> However the suggestion that a dietary factor may be involved is a useful one and some experimental support may be given this hypothesis by the recent work of Himes *et al.*<sup>19</sup> who found that rats on a diet of cereals absorbed more iron than control animals. These authors also suggested that a low level of dietary phosphite might favor iron storage.

Imeh *et al*<sup>24</sup> using radioactive iron found in a case of hemochromatosis that the rate of utilization of radioiron was profoundly depressed but at the same time there was nothing fundamentally wrong with erythrocyte production. They conclude (with additional evidence from animal experiments) that radioiron to some extent measures tissue iron stores and that the utilization of it is inversely proportional to the size of these iron stores. If this be true the method should prove of great value in the clinical diagnosis of hemochromatosis.

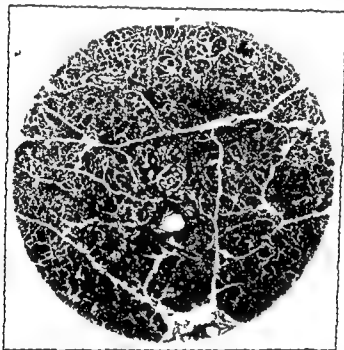


FIG. 97—Hemochromatosis. Moderate pigmentation of pancreas. Note interacinar fibrosis; also practically normal islet in center.

Alper *et al*<sup>25</sup> report that of a total dose of 34 mg. of radioiron given orally to a hemochromatotic patient about 60 per cent was retained. The importance of any of ferritin<sup>26</sup> in the metabolic disturbance of hemochromatosis is yet to be settled. This substance, which is apparently of great importance in the transport and storage of iron, is held to be identical with the hepatic vasodilator substance (VDM) of Shorr.<sup>27</sup>

of diabetes, simple portal cirrhosis of the liver, and... concluded that the three last mentioned diseases all are interrelated. They suggest that in hemochromatosis the sequence of events may be as follows:

cirrhosis of the liver and diabetes may both be produced by an alloxan like substance fibrosis of the pancreas then results from portal hypertension consequent to the cirrhosis and finally hemosiderosis occurs due to abnormal retention of iron derived both exogenously and endogenously. This theory which is based on several rather tenuous assumptions still leaves the defect in iron metabolism unexplained.

However this uncertainty as to the primary etiology in no way interferes with the value of hemochromatosis in throwing light on the pathogenesis of diabetes. In the first place it is of great importance in showing that

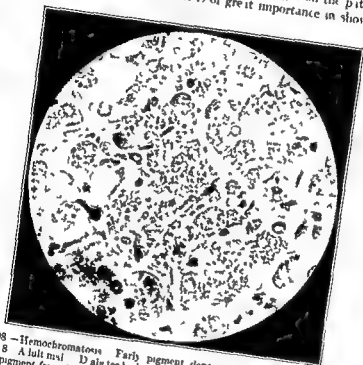


FIG. 98 — Hemochromatosis. Early pigment deposition in islets of Langerhans. No Al<sup>III</sup>. A full mild Diabetes had not developed in this case owing to the presence of many pigment free islets.

perfectly typical diabetes can be produced by a definitely known clinical entity which is known not to be an effect of the diabetic process but to produce it. Moreover the development of the diabetes is apparently directly related to the extent of the involvement of the islets of Langerhans.

Thus Case U23-31 died of cirrhosis of the liver with ascites a perfectly typical case of hemochromatosis. There was no diabetes and while there was a moderate amount of pigmentation of the pancreas nevertheless as can be seen from Figure 97 there is practically no involvement of the is-



Islands. Many normal islands were seen scattered throughout the section. In contrast to this are Figure 92 and Figure 96 both from a case of hemochromatosis with heavy pancreatic involvement and with the development of diabetes.

Another case that of a man aged fifty-two years was of interest owing to his tremendous insulin resistance and will be described in detail in Chapter 21.

Of the 811 pancreases examined in this series 16 showed marked changes characteristic of hemochromatosis and gave the clinical symptoms of diabetes.

The cases are summarized in Table 38.

In one case of hemochromatosis without diabetes we found in the pituitary gland a moderate amount of pigmentation. In two additional cases with diabetes pigmentation was further marked. In three of the recent cases pigmentation has been remarkably widespread in various tissues throughout the body.

TABLE 38.—HEMOCHROMATOSIS

Case No.	Sex	Age	Dural on hemochromatosis yrs	Diabetes mellitus yrs	Liver weight grams	Pancreas weight grams	Skin involved
12271	♂	59	?	1 1	2170	80	+
14910	♀	65	?	3+	2500	45	+
16343	♂	51	1+	0 1	2680	180	+
21531	♂	43	?	0 4	2000	Normal	+
21720	♂	56	?	2 2	1610	—	—
26380	♂	42	?	0 8	—	—	+
A22 115	♂	41	?	0 3	Small	90	+
J 6347	♂	52	4	1 0	Large	—	—
M 2011	♂	53	?	0 1	Small	Normal	—
52490	♂	41	—	1 8	1560	75	+
65066	♂	57	—	1 5	880	Normal	—
80743	♂	53	0 5	7 3	2740	85	+
86811	♂	41	—	4 1	2260	96	—
88667	♂	42	—	5 0	1075	—	—
10737	♂	61	0 1 yr from diag. to time of death	8 0	2600	About normal	+
10710	♂	62	10 0	21 0	1520	50	+

It must be emphasized that the deposition of pigmentation in the pancreas in hemochromatosis is by no means selective for the islands but involves the acinar tissues as well. In other words it is not a specific but a generalized process and the evidence of pancreatic involvement is only of value from the standpoint of the relation of the diabetes to the pancreas rather than evidence in favor of the insular hypothesis itself.

Whatever cell is involved the mechanism is practically the same. According to Mallory<sup>7</sup> hemosiderin appears first then hemosulferin. Others doubt that there is any interrelation between the pigments or the hemo-

fuscin is derived from hemoglobin. At any rate both pigments appear finally the amount of pigment is sufficient to prevent life of the cell. When necrosis occurs some of the pigment is taken up by wandering cells some by the stromal cells. The stroma condenses new parenchymatous cells are formed and new stroma is laid down only for the process to repeat itself.

The hypothesis here suggested that the fibrosis is a direct result of the deposition of the iron pigment is not held by Sheldon<sup>9</sup> who believes that the two processes are independent or by Harbut and Samaki<sup>10</sup> who point out that the only marked fibrosis is found in liver spleen and pancreas other organs often showing heavy deposits of hemosiderin without fibrosis. It is possible however that these differences are essentially quantitative depending on the total amount of iron pigment and the length of time over which it is deposited in a given organ.

As Root and Warren<sup>11</sup> have already mentioned in hemochromatosis frequent evidences of attempts at regeneration can be seen considerable numbers of mitotic figures being found in the island cells.

Case A17-8 a nondiabetic from Dr Mallory's laboratory is of particular interest in that there were a considerable number of pigment free as well as pigmented islands and in the former islands mitotic figures could occasionally be found.

Birker<sup>12</sup> presented a case of much interest in that under insulin therapy which was difficult because of the marked blood sugar variations of the patient swinging easily into hypoglycemia the cutaneous pigmentation diminished.

In a series of 30 cases of hemochromatosis diagnosed during life at the Mayo Clinic 26 showed diabetes.<sup>13</sup>

One of the most interesting (and unexplained) features of hemochromatosis is the high incidence of primary carcinoma of the liver. If one estimates the occurrence by using only the reports in the literature in which 5 or more consecutive cases of hemochromatosis are described one arrives at the remarkably high figure of 18.9 per cent for the incidence of primary carcinoma of the liver in hemochromatosis.<sup>14</sup> The reason for the increased incidence over that of carcinoma in simple portal cirrhosis is unknown.

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## Chapter 21

### THE PATHOLOGY OF INSULIN RESISTANT CASES

From the theoretical standpoint insulin resistant cases are of great interest though their rarity makes their practical importance slight. According to Root <sup>1</sup> a case cannot be considered as falling in this group unless 200 or more units of insulin are needed per day. This figure seems to be generally accepted as an arbitrary definition. Although originally based on probably erroneous assumptions regarding the insulin output of the normal pancreas it has proved convenient in clinical practice. The amount of insulin required in some cases may reach fantastic figures as high as 10 100 units in sixteen hours <sup>2</sup>.

The phenomenon of insulin resistance is a curious one which is often unexplained and many apparently conflicting data exist in the literature as is evident from several recent reviews and summaries of the literature <sup>3 4</sup>. The relatively small number of reported cases is probably a very inaccurate index of the incidence of the condition since many cases are unreported <sup>5</sup>. In view of the theoretical importance of the phenomenon this paucity of cases is unfortunate all the more so since Haunz <sup>6</sup> states that of 27 reported cases only 8 have come to autopsy.

An excellent review of the subject is given by Smelo <sup>7</sup> who points out that although the fundamental mechanism of the hyporesponsiveness is usually not known certain clinical entities are often associated with the condition especially an allergic sensitivity to insulin an endocrinopathy a chronic infection or hepatic disease. The only clear cut mechanism of insulin resistance known is the development in some cases of antibodies to insulin. This mechanism has been emphasized particularly by Lerman <sup>8</sup> and by Lowell <sup>9 10</sup> and more recently by Sherman <sup>11</sup>. Other authors however tend to minimize its importance. For instance Axelrod *et al* <sup>12</sup> found no evidence for an insulin antagonist in their cases by either *in vitro* or *in vivo* methods.

On the basis of a careful analysis of 54 cases collected from the English literature Smelo <sup>7</sup> gives the following classification

Developed In August 1927 on a diet of carbohydrate 78 grams protein 60 grams and fat 123 grams he excreted 40 to 90 grams of glucose in 1

urine daily and diastolic read was present although he was receiving 100 units of insulin daily. In September, 1927, he went into coma and required 525 units daily. Insulin was gradually increased until October 22 when he was receiving 840 units per day. October 27, he was given 1600 units and died in coma October 30, 1927, although he had received 1680 units of insulin in the previous twenty-four hours.

TABLE 19.—CLINICAL STATES ASSOCIATED WITH HYPORESPONSIVE HYPERGLYCEMIA (from Simeloff)

- A Disruption of the functional integrity and innate characteristics of the tissues
  - Hemochromatosis
  - Recurrent jaundice with common duct obstruction
  - Multiple hepatic infarcts
- B Dysfunction of enzyme systems
  - Hypotrophy (lipodystrophy) with hepatic cirrhosis
  - Defective hepatic glycogenesis
- C Dysfunction of hormonal or nervous system regulation
  - 1 Insufficiency of insulin
    - a Delayed absorption
    - b Immunologic inactivation or neutralization
  - 2 Excess of contra-insulin hormone
    - a Pituitary hyperfunction
    - b Acromegaly
    - c Cushing's syndrome
    - d Hemorrhagic cyst of the brain
    - e Hyperthyroidism
    - f Pheochromocytoma
- D Pathologic states of etiologic or concomitant significance not present in known

The essential findings in the post mortem examination were a finely granular dark liver and the pancreas showed marked fibrosis and lipomatosis. Microscopic examination revealed deposits of hemofuscin and hemosiderin in the great majority of the organs and very large amounts in the liver and in the pancreas. There was very little pancreatic tissue present most of it being replaced by fat and fibrous tissue. While all of the epithelial elements of the pancreas were pigmented there were a very few islands which were nearly normal. In the adrenals the cells of the zona glomerulosa were extremely heavily infiltrated with the pigments. Presumably but a very slight amount of functioning islet tissue was present in the pancreas and the liver was very severely damaged. There was sufficient liver damage to explain a very considerable degree of interference with the glycogen-storing function. Indeed liver damage would appear to be the only obvious explanation for the well recognized insulin resistance in hemochromatosis.

Another case while it falls short of the true insulin resistant type is nevertheless very instructive. (24-33) a diabetic woman aged sixty nine years had been a relatively mild case for about ten years. A few months before death there was a marked increase in insulin requirement 50 to 100 units per day being necessary. At autopsy a large carcinoma of the pancreas was found which had destroyed the bulk of the gland. The last 3 cm. of the tail were uninvolved and most of the acinar tissue there had

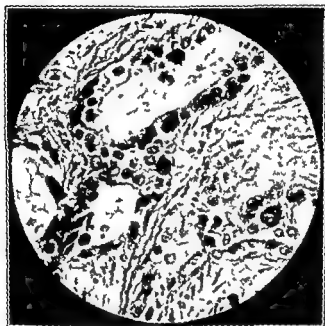


FIG. 33.—Carcinoma of pancreas. Very large insulin requirement. No. 12144. Female aged sixty nine years. Duration of diabetes sixteen years. H&E, fast green acid hematein stain.  $\times 250$ .

atrophied due to duct obstruction while the remaining islands had hypertrophied. Although these islands must have been under considerable functional strain they showed no evidence of hydropic change. Davidson and Fiddleman<sup>8</sup> also report a case associated with carcinoma of the pancreas and present a thoughtful review of the literature on insulin resistance. These cases are of interest in relation to the peculiar and unexplained fact that experimental diabetes produced by partial pancreatectomy is often more severe than that produced by total pancreatectomy.<sup>11,12</sup> One may of course speculate on the possible importance of H G factor produced by  $\alpha$  cells in the surviving part of the pancreas. (See below and Chapter 26.)

Rosen<sup>13</sup> notes that cases with skin lesions may be insulin resistant and suggests that there may be both an allergic factor and a problem of cir-

drate storage. However biopsy specimens of skin from nondiabetic persons examined through the courtesy of Dr A M Greenwood show but little variation in glycogen content between normal skin and skin with various pathological conditions.

Liver injury<sup>13, 14</sup> is one of the most important causes of this phenomenon. While liver insufficiency does not in itself produce diabetes it does profoundly influence a coexistent diabetes. The importance of the liver for the prompt removal of sugar from the blood is well known and obviously any marked interference with liver function would lead to lessened glycogen storage and so decrease the effectiveness of insulin in lowering blood sugar. In certain of the nondiabetic cases of cardiac decompensation with liver damage and of cirrhosis of the liver we have found a smaller amount of intracellular glycogen than that usually encountered. An instance of sudden insulin resistance following thrombosis of the hepatic artery has been reported by Pollack and Long.<sup>15</sup>

In marked contrast is a case of portal cirrhosis cited by Bordley.<sup>16</sup> A male aged forty nine years was a severe diabetic for five years and then developed jaundice followed by cirrhosis in April 1927. In December 1927 the diabetic symptoms disappeared and in April 1928 and in 1930 he showed no evidence of diabetes mellitus.

Interesting observations have been made by Zimmerman *et al*<sup>17</sup> on liver biopsies taken from insulin resistant cases. Many of these have shown large amounts of fat. The authors do not however regard the fatty livers as directly responsible for the insulin resistance but interpret both the diabetes and the hepatic lesion as due to an unknown causative

cortex have long been  
one case improvement  
<sup>18</sup> The experimental

diabetes induced in man by pituitary adrenotropic hormone is characteristically insulin resistant.<sup>18</sup> Ingle<sup>19</sup> in an important review of experimental work has indicated some of the difficulties involved in any attempt to study factors affecting carbohydrate tolerance. Some of his rats treated with adrenal cortical steroids developed tremendous insulin resistance. Insulin resistance is characteristically present in the diabetes associated with adrenal tumors both cortical and medullary (Chapter 16).

The possible importance of the insulin inactivating system (insulinase) described by Mirsky *et al*<sup>20</sup> in cases of insulin resistance cannot be evaluated as yet. This presumed enzyme has been demonstrated in normal rat tissues has been shown to be reduced by fasting and restored by re-feeding but much more work is required to assay its significance.

One of the peculiar features of insulin resistance is the common tendency to spontaneous recovery after a certain period of time. About a third of Simons's<sup>21</sup> cases showed this phenomenon. In the great majority of cases it cannot be attributed to any therapeutic procedure.

The possibility that the hyperglycemic glycogen lytic ( H G ) factor presumably produced by the  $\alpha$  cells of the islets of Langerhans may play a major part in the pathogenesis of insulin resistance has only recently received consideration in the literature. The possibility is mentioned by Lincus<sup>22</sup> but is elaborated particularly by Ferner<sup>23</sup> who theorizes that the essential difference between insulin-sensitive and insulin resistant diabetes lies in a greater stimulation of  $\alpha$  cells in the latter by the diabetogenic factor of the pituitary which he calls the alpha<sub>2</sub> tropic factor (this assumption being apparently based chiefly on the finding of Ham and Haist<sup>24</sup> that the  $\alpha$  cells may be partially degranulated in experimental pituitary diabetes). Such a hypothesis would of course explain conveniently the insulin resistance of experimental diabetes and of the diabetes associated with acromegaly, also the otherwise puzzling finding that total pancreatectomy produces a diabetes requiring less insulin than that produced by partial pancreatectomy. Though based on little available concrete evidence the hypothesis is an attractive one and should stimulate much investigation in the future. Of interest in this connection is the observation that purified growth hormone abolishes the insulin hypersensitivity characteristic of hypophysectomized animals<sup>25</sup>.

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## Chapter 22

### HYPERINSULINISM AND THE PATHOLOGICAL EFFECTS OF INSULIN ADMINISTRATION

**HYPERINSULINISM** may be either functional or organic. Both types are discussed in excellent summaries of the subject of spontaneous hypogly-

following gastroenterostomy in which case the mechanism is apparently rapid dumping of sugar upon the absorbing surfaces of the small intestine with a rapid and excessive rise in blood sugar followed by a precipitous fall (due presumably to overstimulation of the  $\beta$  cells).

The functional types of hyperinsulinism are of no direct import to the pathologist except as regards possible search for hyperplasia of the islands. They are however of great clinical interest since unnecessary surgical explorations of the pancreas have been carried out because of a mistaken diagnosis of adenoma of the islands. Factors to be considered in the clinical distinction of the functional and organic types, the interpretation of the glucose tolerance test, etc., are discussed by Conn<sup>1</sup> and by Marble.<sup>2</sup> On the other hand Perkins *et al.*<sup>3</sup> emphasize the practical difficulties that may be encountered in individual cases in making a definite diagnosis. Conn<sup>1</sup> states that true functional hyperinsulinism is readily controlled by diet (high protein, low carbohydrate) designed to prevent the absorptive rise of blood sugar.

**Organic Hyperinsulinism** — The case which gave the final proof to the origin of insulin from the islands of Langerhans, that of Wilder. Allen

importance to warrant presenting a brief summary of it.

The case was that of a man aged forty years who had had a gastroenterostomy in 1919 and renal colic in 1924. In January 1925 the fasting blood sugar was normal, one specimen of the urine was sugar free while others showed traces of sugar. In October 1926 the patient came to the Mayo Clinic complaining of attacks of faintness and weakness for the past eighteen months. These were accompanied by profuse perspiration and trembling. They usually came on when meals were delayed or if unusual exertion was undertaken. The patient found that they could be prevented by eating between meals. The first attack resulting in collapse was in

November, 1925. The similarity of it to an insulin reaction led to the diagnosis of spontaneous hyperinsulinism. Exploratory operation was performed December 4, the pancreas was found to be large hard and nodular and nodules were present in the right lobe of the liver. The diagnosis of carcinoma of the islands of Langerhans with metastasis to the liver was made. On January 3, 1927 death occurred apparently from exhaustion. The liver was much enlarged weighing 1392 grams. There were five nodules in the liver ranging from 5 to 8 1/2 cm. in diameter. The pancreas weighed 120 grams. The tail was thickened and enlarged and scattered through it were yellowish gray rounded nodules ranging up to 1 1/2 cm. in diameter. In addition there was a small cyst at the extreme tail of the pancreas. Microscopic examination showed the cells to be characteristic of those of the islands as were the nodules in the liver. There was a very large amount of glycogen in the liver cells and in the convoluted tubules of the kidney. Analysis of the liver showed it to contain 8.2 per cent glycogen. Assay of the insulin content of the liver tissue showed that there were approximately 40 units of insulin for each 100 grams of tumor tissue. It is peculiarly interesting that there were large amounts of glycogen in the epithelium of the convoluted tubules of the kidneys in this case of hyperinsulinism.

Closely following this case were the cases of Thalhimer and Murphy\* in which a large nodule of cells resembling those of the islands was found in the pancreas apparently a low-grade carcinoma and of McClenahan and Norris† in which a large adenoma of island-cell type had given rise to hypoglycemic symptoms.

The first case of cure of such a lesion was that of Howland *et al.*‡ A married woman in September 1922 became semiconscious and restless with sweating and vomiting. At the end of an hour she felt fairly well again. Six of these attacks occurred up to 1924 when they became more frequent and lasted longer occasionally for twelve hours. Later on she discovered that a glass of milk drunk at the first sign of an attack might ward it off. The physical findings were essentially negative. The blood sugar during one attack dropped to 0.04 per cent. This attack was promptly relieved by the administration of intravenous glucose. On March 15, 1925 operation was performed and an encapsulated nodule 1 1/2 cm. in diameter fairly well demarcated found in the middle of the pancreas. On microscopic examination the tumor was diagnosed as a carcinoma of the islands of Langerhans owing to the resemblance of the cells to the island type and the presence of scattered mitotic figures. In places the tumor cells directly invaded the surrounding pancreatic tissue. The postoperative course was uneventful and there has been no recurrence of attacks since operation. No evidence of metastasis was noted at the time of operation.

The studies of O'Leary and Womack§ and Whipple and Lantz|| have done much to clarify our knowledge of the island-cell tumors.\*

\* The islet-cell blastoma has been proposed for these tumors by Lothman and insulinoma by Crahan and Tiron.

Frantz<sup>11</sup> points out particularly the difficulty of distinguishing benign from malignant tumors especially as regards encapsulation or the lack of it. It is possible that many of the

About 20 cases of carcinoma of the islets have been reported most of them without satisfactory differential stains<sup>14,15</sup>. The cell type in these tumors is of course of great interest but investigation has been hampered by the lack of adequate techniques for staining the specific granules. Laidlaw<sup>16</sup> using Bensley's acid fuchsin-methyl green found a predominance of  $\beta$  cells in some cases. Duff and Murray<sup>17</sup> reviewing the literature found that a varying proportion of cells stained like normal  $\beta$  cells in the few cases in which satisfactory stains had been obtained. It is probable that some of the newer methods such as the Gros-Schultze silver stain and Gomori's new aldehyde-fuchsin stain (see Appendix) will be of great service in the study of these tumors. In fact Hess<sup>17</sup> using the Gros-Schultze method has already reported an apparently nonfunctioning carcinoma of the islets consisting largely of  $\alpha$  cells.

Terbruggen<sup>18,19</sup> using a slight modification of Bensley's acid fuchsin-methyl green as well as the Gros-Schultze method has demonstrated a few  $\alpha$  cells in functional tumors consisting chiefly of  $\beta$  cells. Terbruggen calls attention to the fact that if one assumes the total weight of islet tissue in the normal pancreas to be about 1 gm. as suggested by Ogilvie's work (see Chapter 2) then an adenoma weighing only 1 gm. would approximately double the insulin producing tissue of the body. He also made a careful study of the remaining pancreatic tissue in his cases and arrived at the interesting conclusion that the ratio of  $\alpha$  to  $\beta$  cells was in the diabetic range i.e. from 1/2 to 1/3. Hultquist<sup>20</sup> also noted a relative increase in silver cells in uninvolved parts of the pancreas from cases of adenoma with hypoglycemia. This observation is perhaps to be correlated with Ferner's<sup>21</sup> experimental work on the guinea pig in which he found a reduction in  $\beta$  cells and an increase in  $\alpha$  cells following repeated injections of insulin. (See Chapter 2). This finding may likewise be related to the common observation that a period of hyperglycemia lasting several days may follow the removal of an adenoma of the islands.

nonfunctioning adenomas using the criteria set up by one of us (S.W.) for the recognition of an adenoma (resemblance in morphology and arrangement of cells to those of normal islands; encapsulation with compression of adjacent pancreatic tissue and a diameter of at least 1 mm.). Meissner made the interesting observation that all the functioning tumors contained hyaline similar to that seen in the islands in some cases of diabetes and the nonfunctioning ones did not (with the possible exception of three ques-

tionable cases). The hyaline was even found in the metastases in some of the malignant tumors. The relation of this substance to amyloid is unknown but Meissner raises the question of whether it may not be related to the secretion of insulin rather than representing a secondary degenerative change.

A remarkably extensive tabulation of no less than 398 recorded cases of tumors of the islands is given by Howard *et al*.<sup>21</sup>

While it is generally assumed that diffuse hyperplasia of the islands may be a cause of hyperinsulinism we are not aware of any case in which a completely convincing demonstration of this mechanism has been given. Sendraul and Bazer<sup>22</sup> cite two cases of biopsy of the pancreas in which they felt that they could demonstrate an increase of  $\beta$  cells without increase in number and diameter of the islands. In such cases it would seem that the whole pancreas must be studied and that the considerations relating to total islet mass cited in Chapter 3 should be taken into account. Relief of symptoms in a patient following subtotal pancreatectomy is of course no reason for assuming the existence of hyperplasia. Careful differential counts of  $\alpha$  and  $\beta$  cells correlated with estimations of total volume of the islands would however be of great interest.

## PATHOLOGICAL EFFECTS OF INSULIN ADMINISTRATION

An abundant literature<sup>2</sup> has grown up which deals thoroughly with the insulin reaction and hypoglycemia. A wide range of symptoms has been reported even to transient attacks of hemiplegia.<sup>23</sup> Little however is known as to the demonstrable anatomical changes.

Fortunately there are but two deaths from overdosage of insulin in the present series.

Wohlwill<sup>24</sup> has described 2 fatal cases in which the brain was examined after death. The first was a woman aged sixty-two years whose diabetes had been present two years. A breast abscess developed. For three days before death which occurred in coma she had received 100 units of insulin daily. Her urine was sugar free and there was no acidosis.

The second case a diabetic aged twenty-nine years whose disease was of one and one quarter years duration was admitted in coma. Insulin to the amount of 135 units was given the first day 70 units the second and 90 the third when death occurred. The blood sugar dropped from 0.462 to 0.005 per cent in twenty-four hours.

Sections of the brain in each case showed a marked anisocytotic appearance of the glia cells. The ganglion cells showed degenerative changes with pyknotic nuclei and loss of normal staining reaction and in the first case the axis cylinders were markedly swollen and irregular.

Cerebral changes have been reported<sup>25</sup> in a case of a woman aged forty-nine years whose diabetes had been present for five years. The patient experienced several insulin reactions after having been on protamine

insulin four months. She was found in hypoglycemic coma and remained comatose in spite of treatment until she died eight days later. At autopsy but little island tissue was found. The brain was edematous with extravasations of blood about the smaller vessels. Much cerebral arteriosclerosis was present.

Wolf's<sup>1</sup> case showed no edema of the brain but minor loss of cortical cells and mild ependymitis. Terplan<sup>22</sup> noted in addition to marked edema of the brain and cord extreme capillary injection and marked destruction of nerve cells especially in the third and fifth layers.

Baker and Lusk<sup>23</sup> ascribe the ganglion cell changes to post mortem alterations and emphasize numerous new and old hemorrhages throughout the brain which they believe are most numerous in those patients with the most severe convulsive seizures.

Cerebral changes were apparent in Case No. 12420, an eight year old boy who died from hyperinsulinism. He had had diabetes for four years. Edema of the brain was marked even to the extent of subependymal blebs and vascular congestion was striking. Case No. 72878, a boy aged sixteen years suffering from pulmonary tuberculosis and diabetes of five years duration had the misfortune to have while in sanatorium a hypoglycemic attack which was diagnosed as coma. Five hundred units of insulin had been given before the mistake was recognized and glucose was then given in a vain attempt to avert death. The liver contained much glycogen, the brain was edematous with capillary congestion and some loss of cerebral cortical cells.

Lawrence *et al.*<sup>2</sup> found extensive nerve cell degeneration and gliosis proliferation in 6 fatal cases.

— — — — — changes in brain tissue  
 were first  
 showed liquefaction and later pericellular encrustation and finally disappeared  
 regarded as the  
 lesions of the  
 produced by thujone, camphor and metrazol.

Hesler and Hunnrich<sup>24</sup> studying insulin hypoglycemia in the dog noted a progressive decrease in brain glycogen occurring (with the exception of the cerebral cortex) from the rostral part caudad.

Hicks<sup>25</sup> points out that the pattern of destructive lesions of the brain

such simple formula as the richness of the diet supply is not sufficient. Other factors especially the individual metabolic patterns of different strains may be important for instance the fact that the consumption

Hicks found that in rats given large doses of insulin the cerebral cortex and corpus striatum were damaged sometimes severely but the corpus callosum substantia nigra and cerebellum were never involved. The neurons of the fascia dentata as well as the pyramidal layer of the hippocampus were extensively necrotic in several animals. The thalamus showed rare necrotic lesions in some instances. There was no strict correlation of dosage and brain damage but some of the most severe lesions followed single bouts of hypoglycemia.

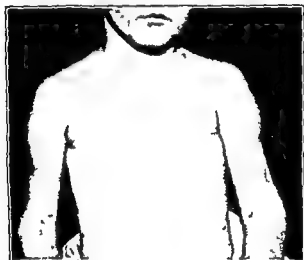


FIG. 100.—Boy five years of age. Duration of diabetes 18 years. Note bilateral atrophy of subcutaneous tissue in regions where insulin had been injected. (Clinical condition at nutrition goal.)

While the changes ascribed to hyperinsulinism or better to extreme hypoglycemia are not very characteristic there seems to be ample justification in making the diagnosis of changes compatible with hyperinsulinism. When combined with these there is definite clinical evidence of hyperinsulinism with chemical evidence of hypoglycemia the diagnosis may be definitely established. From histological findings alone such a diagnosis is not justifiable.

However tremendous amounts of insulin may be given without apparent ill effect in certain cases. A striking example is Joslin's Case No. 6247 reported by Root<sup>29</sup> and cited in Chapter 21 as an example of insulin resistance in which 1650 units daily failed to prevent the development of coma and no changes referable to the insulin were found post mortem.

Occasionally local changes are noted in the skin at the site of insulin injections. In the first few months of insulin production painful nodules

were rarely noted in the skin of injected patients developing soon after injection and persisting for some weeks. These were apparently due to the type of antiseptic then in use and are never seen if present.

Adlersberg<sup>10</sup> has reported multiple skin nodules considered to be lipomas developing in a girl twenty years of age who had been injecting insulin six years.

**Insulin Lipodystrophy**—Localized atrophy of the subcutaneous fat is due to repeated injections of insulin in the same area. Barborka<sup>11</sup> and Depisch<sup>12</sup> have reported several cases. The depressed character of the lesions which range from 2 to 9 cm. in diameter and are about 1 cm. in depth and the absence of any evidence of inflammation make their appearance very characteristic. Occasionally these foci may be painful. Isaac<sup>13</sup> suggests a small amount of lipolytic ferment may be mixed with the insulin in such instances. This fails to explain most cases. Trauma to fat is the most likely explanation. Recovery may occur.

The experimental observations of Renold *et al.*<sup>14</sup> may have some bearing on this phenomenon. These investigators found that insulin would increase the deposition of glycogen in fat cells to a significantly greater extent in diabetic rats than in normal animals and that insulin had a direct local effect on the adipose tissue at the site of injection causing the greatest increase in glycogen in this region.

**Hypersensitivity**—Hypersensitiveness to insulin was noted<sup>15, 16</sup> not infrequently in the early years of its use before the foreign proteins had been adequately removed. These reactions usually took the form of serum sickness although an occasional case of true anaphylaxis has been reported. Insulin allergy was noted in 10 per cent of patients at the Mayo Clinic.<sup>17</sup> Gastrointestinal hypersensitivity has been noted.<sup>18</sup>

The clinical importance of hypersensitiveness to insulin is extremely small. The symptomatology is usually mild and the patient for

While insulin hypoglycemia may have a damaging effect on the heart unimpaired by coronary disease perhaps through a stimulation of adrenal medulla.

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## Chapter 23

### INFANTS OF DIABETIC MOTHERS

It has been known for many years that there is a high incidence of stillbirths and of neonatal deaths in the pregnancies of diabetic women.<sup>1-3</sup> This holds true for both the preinsulin and insulin eras, although there has been a striking improvement in recent years. The causes are obscure and post-mortem examination of the infants has done little to shed light on the problem. Certain interesting findings may, however, be noted and recorded particularly the resemblance of these infants to those suffering from erythroblastosis fetalis,<sup>10</sup> and the influence of the diabetic and prediabetic state on the size of the baby<sup>11-15</sup> and on certain tissue changes.



FIG. 101 — Typical post-natally treated) The placentomegaly are well shown

The information on which this chapter is based is largely derived from a study of 50 autopsies on infants of diabetic mothers under the care of Drs Priscilla White and Raymond Titus performed at the Faulkner Hospital\* from 1937 to 1949 inclusive.

In the latter part of this period the majority of the mothers had been maintained on the hormonal regimen described by White<sup>1-3</sup>. Of the 50 infants, 25 were delivered by Cesarean section.

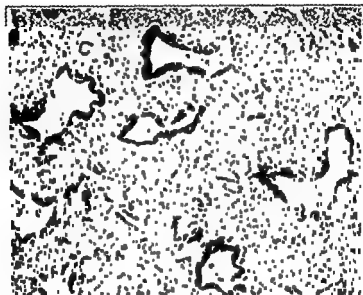


FIG. 102 Hyaline membrane (vernix caseosa) lining alveoli in lung of infant of diabetic mother.

TABLE 10 CAUSES OF DEATH

Undetermined (including 11 macerated still births)	18
Prematurity (under 32 weeks or 1500 gm.)	14
Aspiration of amniotic fluid	8
Birth injury	4
Pneumonia	4
Congenital anomalies	3
Adrenal hemorrhage	1

50

**Causes of Death.** In the accompanying table (Table 10) are listed the causes of death in the 50 cases. A few words of explanation are in order since the causes listed in the table are, in many instances, not the same as

\* Practically all of these autopsies were done by or under the direction of Dr. J. B. Hazard, Dr. G. K. Mallory, or Dr. P. M. LeCompte.

those recorded in the protocols. For instance a number of cases originally signed out as pulmonary atelectasis or prematurity are here classed as undetermined since pulmonary atelectasis *per se* is not considered a satisfactory cause of death and prematurity was arbitrarily defined as noted below. The undetermined classification also comprises all of the 11 stillbirths in the series since no adequate cause of death could be assigned to any of them (evidence of intrauterine anoxia was not convincing). Prematurity was arbitrarily defined as present whenever a gestational age of thirty two weeks or less or a birth weight of 1500 gm or less was recorded. Death was attributed rightly or wrongly, in 8 cases to aspiration of amniotic fluid the chief criterion being the presence in all of a well marked vernix membrane in the lungs (See Fig 102 and discussion below). The birth injuries were, in all instances meningeal tears. The anomalies which were sufficient to cause death in three instances consisted of 2 congenital cardiac malformations and one cephalo meningocele.\*

**Body Weight and Body Length.** Microsomia is perhaps the most commonly noted finding in the infants of diabetic mothers. Kraus<sup>21</sup> gives the following tabulation of weights of 608 infants of 155 diabetic women.

Normal children	385 (63.3%)
4 kg children	110 (18.1%)
4½ kg children	47 (7.7%)
5 Kg children	49
5 to 6 Kg children	17 (10.8%)

The large size of these infants has been emphasized particularly by Miller who has also called attention to the fact that apparently normal women who have excessively large babies are likely to develop diabetes in later life.<sup>11, 12, 13</sup> This extremely interesting complication of the prediabetic state has been confirmed by others.<sup>2, 22</sup>

The observed weight in 43 cases was compared with the calculated weight according to the recorded gestational age.<sup>24</sup> These calculations were based on the empirical formulæ of Scammon and Calkins<sup>25, 27</sup> which are presented in graphic form by Dunham<sup>28</sup> and which compare fairly well with the data of Ylpo.<sup>29</sup> (These formulæ are for fetal dead weight which according to Scammon and Calkins<sup>25</sup> averages about 200 gm less than the live weight.) Of the 43 babies only 10 were less than the calculated weight and most of these were under 1500 gm. Of 30 infants weighing over 2000 gm at birth only 2 were less than the calculated weight. Three infants exceeded the calculated weight by less than

40 gm 14 were from 400 to 1000 gm heavier and 10 or more than a third exceeded the calculated weight by over 1000 gm

Similarly the *observed length* was compared with the calculated length<sup>2</sup> Of 40 infants for whom data were available 13 were less than the calculated length one was the same two were from 0 to 2 cm longer five were from 2 to 3 cm longer and 18 were over 3 cm longer Five babies were heavier but shorter than the calculated standards and two were lighter but longer (both of these however were under 1000 gm)

**Prematurity** — One striking fact which emerges from the data is that the relatively large size of most of these babies is extremely deceptive and that to judge from the menstrual history the great majority of the babies (at least in this series) must be regarded as premature Standards for judging prematurity are of course arbitrary but commonly accepted upper limits are thirty-eight weeks of gestation 2500 gm body weight and 47 cm body length<sup>3</sup> In the present series 42 of 47 babies were at less than thirty-eight weeks of gestation when born according to the menstrual history and on the same basis the *calculated weight* of 38 out of 42 would be less than 2500 gm and the *calculated length* in 34 of 40 would be less than 47 cm Admittedly this conclusion necessitates placing considerable reliance on the menstrual history but as stated above this is thought to

centers are not available (See below)

**Heart** — Among the organs the heart occupies a position of major importance because of previous observations particularly by Miller<sup>2,4</sup> that these infants may show a considerable cardiac enlargement In trying to determine what may be considered a normal heart weight for the newborn and especially for prematures we encountered the same difficulties recorded by Miller<sup>2</sup> as regards variations in published data After inspection of a number of such figures it was decided that the formula devised by Miller<sup>2</sup> would be satisfactory especially since his calculations seem to give if anything a slightly lower figure than some others Miller's formula is as follows

$$x = 0.00021x^2 + 0.63 + 0.327$$

where  $x$  is the weight of the heart and  $x$  the body weight in grams

Application of this formula to the present series yielded the following results { of 41 infants of diabetic mothers 19 (or 44 per cent) had hearts which exceeded the calculated weight by more than twice the standard deviation Out of a control group of 70 infants only 6 (or about 9 per cent) had hearts exceeding the expected value by more than twice the standard deviation } It seems likely then that nearly half of the infants in the present series had significant cardiac enlargement { It is of interest that 2 of

3 infants with erythroblastosis fetalis in the control group had heart weights exceeding the expected figure by more than twice the standard deviation. Considerable amounts of glycogen have been demonstrated in the hearts of infants of diabetic mothers chiefly by the rather unreliable Best carmine staining technique<sup>19,20</sup> and the hearts of diabetics in general appear to contain more glycogen than normal<sup>21</sup> (see Chapter 5). A marked vacuolization of the cardiac muscle fibers seen best in cross section is a common finding in these infants often appearing more striking than that of the normal fetal heart which also contains a good deal of glycogen.

Since it seemed possible that the glycogen content might account for at least some of the apparent enlargement and might also account for some of the circulatory and respiratory difficulty observed in these babies, chemical analysis for glycogen was carried out (courtesy of Dr. Albert Renold) on five hearts from infants of diabetic mothers and one heart from an infant of a nondiabetic mother.

TABLE 41—GLYCOGEN CONTENT OF INFANT HEART

Infant No.	Hrs post death	Heart	Liver	Muscle
A 49 1	4	3.52%	4.81%	7.32%
A 49 13	3	0.26%		
A 49 20	~1	0.024%		
A 49 23	5½	0.188%		
A 50-73		1.38%		
A 50 36 (nondiabetic)		1.14%		

Each heart was frozen solid shortly after removal from the body and kept so until the analysis was carried out. The results are shown in Table 41. It is recognized of course that figures such as these are of dubious value in view of the time that elapses between death and necropsy due to the necessity for obtaining autopsy permission etc. Cruchshank<sup>22</sup> gives average figures of 0.60 and 1.25 per cent for normal and diabetic hearts respectively. According to this two of the hearts would be distinctly in the diabetic group.

The reasons for the remarkably high content in A 49 1 exceeding the values in some cases of glycogen storage disease<sup>23</sup> are not it would seem. At least one infant of a diabetic mother has been reported as an example of glycogen storage disease.<sup>24</sup>

**Lungs**—The gross and histologic findings in the lungs do not appear to differ essentially in most cases from the picture in normal controls. Atelectasis of varying degree was present in every case but the lung structure resembled that seen in control cases of stillbirths and neonatal deaths. No instances of alveolar dysplasia as described by McMahon<sup>25</sup> were seen. The amount of amniotic fluid in the lungs seemed greater in many cases than in comparable controls. In 8 instances of neonatal death (not stillbirths) the presence of a thick pink-staining hyaline liver often giving a

faintly positive Sudan stain (vernix membrane) plastered on the lining of the occasional open alveoli (Fig. 102) seemed sufficient for a cause of death. It is hard to conceive of much diffusion of gases through such a layer<sup>38</sup> and the membrane itself must tend to increase the cohesiveness of the alveolar surfaces and thus prevent neighboring alveoli from opening. Although most of these cases were delivered by Cesarean section, one of the most severe was a spontaneous delivery, hence it seems unlikely (although it is still possible)<sup>38, 39, 40</sup> that the mode of delivery is a major factor in promoting aspiration of amniotic fluid.

The postulation of this cause of death is admittedly an assumption and a guess. It seems to be generally agreed that there is a tidal flow of amniotic fluid in the larger air passages of the fetus *in utero* and that more or less vigorous respiratory movements may occur before birth.<sup>41, 42</sup> Certainly traces of amniotic fluid in the form of cornified epithelial cells may be found histologically in the lungs of practically any stillborn or newborn infant. However a well marked vernix membrane can hardly be neglected as a possible cause of respiratory embarrassment. The application of suction<sup>43</sup> to the new-

born hyaline membrane does not represent vernix or the result of aspiration of amniotic fluid but rather a reaction to injury analogous to the membrane seen in adults in the pneumonia associated with pandemic influenza or in so-called atypical pneumonia. They found the membrane almost confined to premature infants dying within forty-eight hours of birth and coming from mothers whose pregnancy and delivery were uneventful. More recently Miller *et al.*<sup>44</sup> have modified this view and apparently hold that the membrane is a manifestation of pulmonary edema which they think may be related to dysfunction of the vagus nerves since they have produced something similar in animals by section of the vagi. In both animal and human material they believe they have demonstrated a polysaccharide in the hyaline membrane.

Dick and Pund<sup>45</sup> believe the membrane to be formed of vernix but suggest that the vernix must have a high fat content; in this way they explain the absence of the membrane in some cases in which it would be expected to be present.

Bruns and Shields<sup>46</sup> hold, with Miller, that the hyaline membrane is not due to aspiration of amniotic fluid. They point out the variety of conditions in which such a membrane has been observed both in human and experimental material and emphasize the possibly important fact that a similar membrane can be produced experimentally by high concentrations of oxygen, thus raising the disturbing question of whether overdulous inhibition therapy may not predispose to formation of the membrane.

Blyskal *et al.*<sup>47</sup> on the basis of 500 autopsies and some experimental work hold that the membrane is composed of the concentrated protein of

amniotic fluid that it is most common in premature infants and in more mature infants with a history of a complication such as Cesarean section and that it is a major cause of death in the neonatal period.

Obviously much more work must be done on this crucial question.

**Spleen**—The weights of the spleens were compared with the expected figures for the observed body weight using the table of Potter and Adair<sup>18</sup> which gives weights for various organs in terms of body weight. In 41 cases the following results were obtained: in 19 cases the observed weight of the spleen was less than the expected weight; in 7 cases it was the same

questioned whether the figures are significant in view of the wide variations in organ weights that may be encountered.<sup>19</sup> It seems possible that if any increase of weight were present it might be accounted for on the basis of islands of erythropoiesis which in some cases seemed more abundant than would be expected for normal infants of the same gestational age.

**Liver**—Again the figures of Potter and Adair<sup>18</sup> were used as standards. In 42 cases in which adequate figures were available the results were: 11 were from 0 to 25 gm. over the expected weight; 15 were more than 25 gm. over the expected figure; and 8 of these were more than 40 gm. overweight. In the control group of 20 cases 21 were from 0 to 25 gm. over the expected figure; 7

40 gm. or

infants in

these cases since the great majority were premature infants (see above)

pancreas by eosinophilic leukocytes.

Hyperplasia of the islets was considered to be present in 23 of 27 cases. In a control group of 20 cases hyperplasia was found in 3 cases. (It is noteworthy that 2 of these 5 were cases of erythroblastosis fetalis; further discussion of this point follows below.) Since actual measurement of islets requires serial sections of the glands, conclusions as to presence or absence of hyperplasia were based on simple inspection combined with measurements made with a micrometer eyepiece. The great majority of the cases regarded as hyperplastic exhibited islands which exceeded the 200 micr. diameter suggested by Potter et al.<sup>20</sup> as the approximate upper limit of normal.

number of  
larged hyperchromatic nuclei noted

In many instances the hyperplasia was so extensive that entire low power microscopic fields composed solely of islands of Langerhans could be found (Fig 103). Frequently the hyperplastic islets contained enlarged hyperchromatic nuclei. In a few cases there was striking evidence of the formation of islets from small ducts, the ducts sometimes being incorporated within the islet (Fig 104). The great majority of the cells making up the large islets seem to be  $\beta$  cells, as could be shown in a few favorable cases with Gomori's chrome alum hematoxylin stain and as Hultquist *et al*<sup>11</sup> have shown with the Grob-Schultze stain. The  $\alpha$  cells appear to be present in approximately normal numbers, the increase in size of islets being due exclusively or almost so to proliferation of  $\beta$  cells.

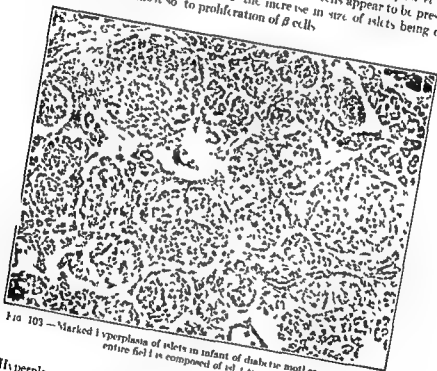


Fig. 103 — Marked hyperplasia of islets in infant of diabetic mother. Almost the entire field is composed of islet tissue.

Hyperplasia of the islets of Langerhans in the infants of diabetic mothers has been recognized for some years.<sup>10,12-15</sup> However, it does not occur in all such infants, nor is it specific for maternal diabetes, as Potter *et al* have pointed out.<sup>16</sup> The cause of the hyperplasia has usually been considered to be the maternal hyperglycemia with consequently increased demands for insulin. Indeed, it has been shown that the severity of the mother's diabetes may be reduced during pregnancy only to return to its former state after delivery, thus suggesting that the insulin of the fetus is being used by the mother.<sup>17,18</sup> Also, experimental work suggests that certain





FIG 104 —Hyperplastic islet of Langerhans in infant of diabetic mother. Small ducts are included within the islet, and some acinar tissue is intimately associated with one end. Some nuclei are large and hyperchromatic.

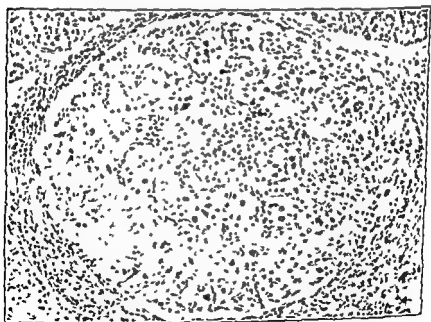


FIG 105 —Hyperplastic islet of Langerhans from infant of diabetic mother. The leukocytes within and around the islet are eosinophils.

adult animals, e.g. rat<sup>11</sup> are capable of hyperplasia of the islets in response to hyperglycemia and that the young of other species may have the same ability. Evidence against hyperglycemia as the stimulating factor in islet hyperplasia is however embodied in Walker<sup>12</sup> who claim that the infants of prediabetic women with normal blood sugars may show the same changes.

Of great interest is the previously described hyperplasia of the islets of Langerhans which occurs in some (not all) cases of erythroblastosis fetalis<sup>13</sup> a plausible explanation of this phenomenon seems to have been offered.

A striking change which may be found in the pancreases of infants of diabetic mothers is an intense infiltration of eosinophilic leukocytes. These are found usually in the interstitial interlobular tissue but are often interplastic islets (Fig. 105). More or less eosinophilic infiltration was noted in 12 cases. This phenomenon has been noted by others<sup>14</sup> and was described in the last edition of this book as representing extramedullary hematopoiesis. It may well be such although the presence of large masses of eosinophils and particularly of what appear to be mature eosinophilic leukocytes in some cases suggests an infiltration or exudate. In other instances the cells resembled eosinophilic myelocytes more suggestive of extramedullary hematopoiesis especially since similar cells are often found elsewhere particularly in the interstitial tissue of the thymus in both the present series and in normal control infants.

**Gastrointestinal Tract**—Nothing of note was found in this system present series and in normal control infants.

**Adrenals**—Particular attention was paid to the adrenal glands in view of current interest in their relation to carbohydrate metabolism and in view of the fact that they have been stated to be enlarged in the infants of diabetic mothers.

It soon became apparent that any attempt to form judgments concerning the weight of these glands was futile. Not only were the published normal values for the weight of these glands (in women<sup>15</sup> & much smaller<sup>16</sup>) found to vary tremendously but when Scammon's formula for the weight of these glands was applied to this and a control series the results shown in Table 42 were found.

TABLE 42 WEIGHTS OF ADRENAL GLANDS IN INFANTS

Diabetic mothers Controls	Less than calculated	Name as calculated	Overweight 5% less than 3 X 5 D	Over 1 X 5 D	Total
	12	1	2	12	30
	11	2	8	19	42

In almost half of both the diabetic and control series the adrenals exceeded the calculated weight by more than three times the standard value. It seems obvious that in conclusions can be drawn.

A series of about ten adrenals from infants of diabetic mothers and ten control cases were studied in frozen section by some of the histochemical techniques in current use for the demonstration of ketosteroids (*i.e.* staining with Sudan III and examination of sections treated with acetone and of untreated sections by polarized light). No significant differences between the two series were noted. There appeared to be no difference in the x zone or androgenic zone<sup>67 68 69</sup> in those infants who had lived more than a few hours; the expected evolutionary changes<sup>70</sup> were found to be beginning in this region in both groups.

The general lack of distinctive morphological findings in the adrenal appears to correlate well with the results of eosinophil counts done on about a dozen of these infants at the Faulkner Hospital.<sup>71</sup> Counts were done as soon as possible (usually ten to twenty minutes) after delivery by Cesarean section also at four hours, one, two, three, five and seven days after delivery. The data when compared with a small control group of Cesarean sections on nondiabetic mothers showed no apparent significant differences. Infants of both groups were usually born with relatively high counts (around 600 or more per cu. mm.) then showed a sharp drop (usually less than 50 per cent, however) at four hours, then a maximal fall to around 100 to 200, sometimes less at twenty-four hours, followed by a rise the next day and more or less stabilization at a level distinctly lower than that found at birth.

**Kidneys**—Kidney weights were compared with calculated normals based on the figures of Potter and Adair.<sup>72</sup> Of a series of 39 cases 16 were less than the calculated weight, 9 were the same (*i.e.* within 1 gm.) and 14 were over the calculated value. Only two instances of marked enlargement (*i.e.* more than 30 per cent over the expected weight) were encountered, and in these cases the accuracy of the figures may be questioned. No significant trend was apparent.

Glycogen was demonstrated apparently in the loops of Henle by the Best carmine method in two cases in which the maternal blood sugar had been high just before delivery (Fig. 106). This observation would seem to be further evidence for a high fetal blood sugar reflecting maternal hyperglycemia and would also seem to reinforce the hypothesis that so-called glycogen nephrosis is simply the result of an elevated blood sugar. Hinde<sup>73</sup> reports glycogen in the collecting tubules in the normal newborn of several species of mammals including man. However in the few normal infants that we have examined with this point in mind the glycogen has not appeared to be so sharply localized as in the infants of diabetic mothers.

Potter and Thierstein<sup>74</sup> have indicated their belief that the structure of the fetal glomerulus is a valuable criterion for estimating the age of the fetus. After a study of the kidneys of 1000 infants they concluded that immature glomeruli were rarely seen over thirty-five weeks of gestation in those weighing over 2½ lb. in those measuring more than 40 cm. in length. In general at glomerular maturity

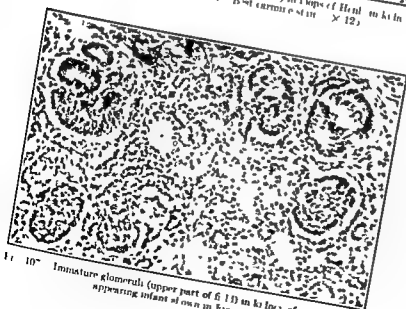
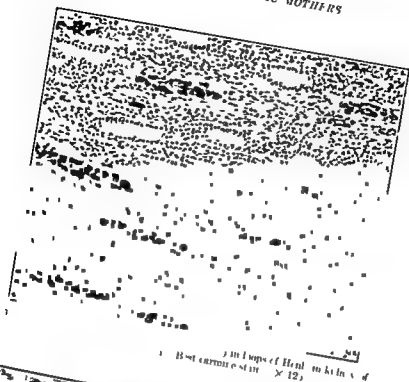


Fig. 10. Immature glomeruli (upper part of fig. 11) in kidneys of premature appearing infant shown in Figure 101

# INFANTS OF DIABETIC MOTHERS

seemed to be more closely related to the size of the fetus than to its gestational age. In a few cases however, the reverse was true.

In Table 43 are listed the data on 4 large infants in this series in whom immature glomeruli were found. Their gestational age was around thirty

TABLE 43—LARGE INFANTS SHOWING IMMATURE GLOMERULI

Infant No.	Gestation at age, weeks	Weight gm	Length cm
A 48 58	36	4200	51
A 49 1	34	3500	51
A 49 13	35	3080	49
A 49 15	34	3460	50

five weeks but all weighed well over 2500 gm and measured 49 cm or more in length (See also Fig 107). If the conclusions of Potter and Thierstein are correct these data provide further evidence for regarding some of the infants of diabetic mothers as large prematures rather than postmature infants. Others<sup>20</sup> also feel that these infants should be treated as premature

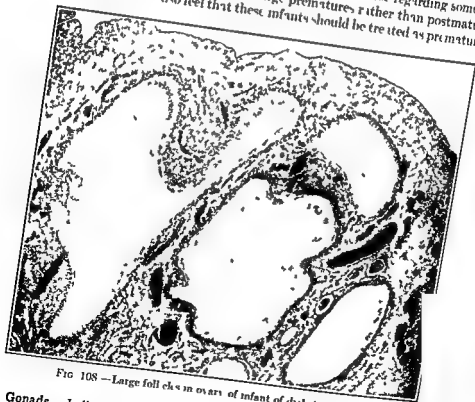


FIG 108—Large follicles in ovary of infant of diabetic mother

**Gonads**—Follicular cysts lined by granulosa cells and surrounded by theca lutein cells have been described<sup>26, 27</sup> in infants of diabetic mothers and have been attributed<sup>28</sup> to the high concentration of chorionic gonadotropin

which is said to occur during part of the gestation period in these women.<sup>5, 7, 74, 75</sup>

Follicles with granulosa cells and theca lutein cells were found in 3 of 10 female infants in this series (Fig. 108) and in at least one of these no hormone therapy had been given to the mother. In one infant a striking decidual reaction of the endometrium was encountered (Fig. 109). In this case however no follicle cysts or corpora lutea were found in the ovaries. The mother had received large doses of estrogen and progesterone. The possible effect on the fetal gonads of the large doses of hormones which

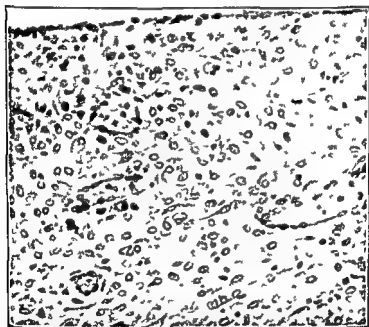


Fig. 109—Decidual reaction in stroma of endometrium. Infant of diabetic mother.  
 $\times 250$

many of these women received is difficult to assay, especially since so little is known on the subject.<sup>76, 81, 82</sup> In two male infants vacuolization of the prostatic epithelium possibly attributable to massive doses of estrogens given to the mother was observed. Such a finding is not however reported by Davis and Potter.<sup>83</sup>

The presence of Graafian follicles in the ovary must be interpreted with caution since in many cases the infants of nondiabetic women may show them.<sup>84</sup> The incidence in the diabetic series does appear to be higher however. An alternative explanation for their presence may be found in the work of Abel.<sup>85</sup> This investigator showed that large doses of insulin in

adult female mice led to marked follicular development and eventually to the appearance of numerous corpora lutea. If it be granted that the hyperplasia of the islets of Langerhans in these babies reflects an overproduction of insulin a possible effect on the ovaries may be considered although Abel apparently obtained no definite results in immature animals.

**Other Endocrines**—No changes considered significant were found in the thyroid parathyroid or pituitary glands. In view of the technical problems and the scarcity of data on normal values for infants no attempt was made to count acidophil and basophil cells in the pituitary. In some instances the acidophil cells did show an apparent predominance as noted by others<sup>25, 26</sup>. Some difficulty was encountered in judging the number of basophils since in some cases both basophils and chromophobes seemed to take a similar dull blue color with Mallory's aniline blue stain.

**Thymus and Lymphoid Tissue**—In histological examination of the lymphoid tissues in newborn infants it is common to find more or less shrinkage of the lymphocytes with pyknosis and fragmentation of their nuclei. This change is usually most obvious (and apparently appears first) in the medulla of the thymus but when well advanced may be found in the cortex of the thymus spleen lymph nodes and other foci of lymphoid tissue as in the intestines.

Particular attention was paid to this phenomenon in the present series in view of recent work on disintegration of lymphocytes by adrenal cortical steroids<sup>27</sup> since it was considered that such an appearance might reflect the presence of an intrauterine alarm reaction<sup>28</sup> in these infants. It was found however that no such conclusion could be drawn. Shrinkage of lymphocytes was invariably absent in the stillborn infants and was commonly present in those who lived more than a few hours being most extensive as a rule in those who survived several days. Comparable results were obtained in the control series. It thus appears probable that the disintegration of lymphocytes whatever its cause is a phenomenon of extrauterine life.

**Bones**—No distinct differences between diabetic and control series were noted in sections of bone (usually rib or vertebra). No adequate roentgenological investigation of density ossification centers etc. has been carried out.

**Placenta**—The condition of the placenta was of particular interest because of the fact that premature aging of this organ has been postulated<sup>29</sup> as the cause of the sharp drop in urinary chorionic gonadotropin observed in the latter part of pregnancy in diabetic women. A series of 54 placentas

series of 53 placentas from nondiabetic mothers.

No definite differences in gross appearance between the two series were made out. However when the weights were compared calculating the amount of placenta per kilogram of baby weight the diabetic placentas

were found to be definitely heavier, the mean for this group being 195.5 gm per kg baby weight as opposed to 165.5 gm for the control group.

this figure. A few of the relatively heavy placentas belonged to quite premature infants, weight 3 to 5 pounds at birth but the majority belonged to infants weighing 6 to 8 pounds.

The two series were not strictly comparable in other respects for instance half of the diabetics were delivered by Cesarean section (hence may have had slightly more blood in the placenta) while practically all the controls were normal spontaneous deliveries. Nonetheless there seems to be a significant difference between the two series. Perhaps the heavier placenta in the diabetics may prove to be another manifestation of immaturity (see below).

It would seem that the sharp drop in chorionic gonadotropin observed in diabetic women<sup>17</sup> might be reflected in a difference in lipid content between the diabetic and normal placenta. Accordingly we have applied some of the methods of Wislocki and Bennett<sup>18</sup> and Dempsey and Wislocki<sup>19</sup> (chiefly Sudan staining and polarized light) to several diabetic placentas and several normal ones. No definite differences could be made out between the two groups. Others<sup>20</sup> have found no characteristic changes in the diabetic placenta.

However McKay<sup>21</sup> reports in a series of these placentas fixed within a few minutes after delivery by Cesarean section the finding of a definite stromal edema of the larger villi. This was quantitatively quite variable sometimes involving the majority of the villi sometimes only a few. This edema disappeared when the placentas stood at icebox temperature for a few hours. McKay suggests that the edema may be simply a manifestation of immaturity of the placenta but this is not yet established. Another change noted occasionally in these edematous villi was the presence of mitotic figures in cells of the cytotrophoblast which was quite prominent in some of the large villi. Preliminary histochemical results indicate that ketosteroids are absent from the trophoblast of these placentas.<sup>22</sup>

**Congenital Anomalies**—It has been generally stated that congenital anomalies are more common in infants of diabetic mothers.<sup>23</sup> This appeared to be true in the present series in which 9 of the 50 infants presented congenital defects summarized in Table 44.

TABLE 44—CONGENITAL DEFECTS IN 50 INFANTS OF DIABETIC MOTHERS

Cardiac	3
Cephalo-meningocele	1
Cystic kidney	1
Absence of one kidney	1



One of the infants with a cardiac anomaly also had multiple skeletal defects. It is interesting that the commonest anomaly was a small inter-ventricular septal defect, this being the sole anomaly in 4 of the 6 cardiac cases. Congenital defects were considered to be the cause of death in 3 cases (2 cardiac and 1 cephalo meningocele).

**Experimental**—The experimental investigation of the problem of fetal mortality in diabetic pregnancies has been fraught with difficulty. Diabetes induced by alloxan in pregnant animals of various species<sup>22-25</sup> has been shown to result in a high incidence of intrauterine fetal death and of abortion but oversized fetuses have apparently not been produced by means of alloxan. Hultquist<sup>26</sup> however in a careful study using partial pancreatectomy during pregnancy was able to obtain giant fetuses in rats. These giant fetuses showed hyperplasia of most of the endocrine glands (thyroid and ovaries were not enlarged and there was only questionable hyperplasia of the islets of Langerhans). The pituitary glands of these large fetuses were said to show increased vascularity and enlarged cells with prominent nucleoli basophilic staining predominating. In further experiments Hultquist and Engfeldt<sup>26</sup> were able to produce giant rat fetuses by administration of various hormones particularly growth hormone to the mothers during pregnancy. The changes in the endocrine glands of these fetuses were said to be similar to those previously obtained when the mothers were subjected to partial pancreatectomy. The authors emphasize the fact that these giant offspring were obtained without prolongation of pregnancy in contrast to certain other experiments which they cite where large postmature fetuses were obtained by artificial prolongation of pregnancy. The mechanism by which growth hormone may act to produce giant fetuses is still to be elucidated. Large fetuses were also obtained but to a lesser extent when chorionic gonadotropin or thyrotropic hormone was given.

**Summary**—The cause of death in the majority of the infants of this series even those who were not early prematures is unfortunately not clear and the reason for the high intrauterine and neonatal death rate remains obscure.

It seems clear however that as a group the infants of diabetic mothers present certain characteristic findings most of which may be expected to be present in babies born in the latter part of gestation but some or all of which may be absent particularly in early prematures. These findings are as follows:

(1) *Macrosomia*—It is a clear-cut and widely recognized fact that the babies of diabetic mothers tend to be larger than normal. The tendency for this large size to obscure actual prematurity has been mentioned above. It seems quite as justifiable and probably much more useful to regard these babies as large prematures as to call them postmature. The evidence provided by glomerular immaturity noted above may perhaps be cited as positive evidence in this regard. The common tendency to use size alone

as a criterion of maturity<sup>27</sup> is certainly open to question. Excellent discussions of the difficulties of establishing a diagnosis of postmaturity are given by Ballintyne and Browne.<sup>28, 29</sup>

The hypothesis that the large bodily size of the infant is due to overactivity of the maternal pituitary—a particularly attractive theory in relation to the prediabetic state—is not as yet supported by adequate evidence.

(2) *Visceromegaly*—Enlargement of the heart is seen frequently enough to be considered significant. Although it is probable that these hearts contain more glycogen than normal ones, the increased size can hardly be due to the glycogen deposit unless one assumes that glycogen may make up more than 10 per cent of the weight of the heart. Enlargement of the spleen and liver is seen in only a fraction of cases and is not clearly significant. It may be due in part to relatively large amounts of hematopoietic tissue although it is not clear that the latter is actually increased out of proportion to the age of the fetus (*see below*).

(3) *Pancreas*—Two changes are found often enough to be considered significant, (a) hyperplasia of the islets of Langerhans and (b) infiltration of eosinophilic leukocytes. The causes of both of these phenomena are obscure.

(4) *Hematopoiesis*—Extramedullary hematopoiesis (chiefly erythropoiesis) especially in the liver and spleen has been cited as characteristic of these infants. As noted above the significance of this finding is questionable when one considers the gestational age of the fetus. If it is truly increased the cause is not clear.

(5) *Glycogen Nephrosis*—Deposition of glycogen in the renal tubules may be expected when there has been an unusually high maternal blood sugar shortly before delivery. However as noted above the specificity of this finding in the newborn may be questioned.

(6) *Gonads*—Growth of follicles in the ovary may be found in a certain percentage of cases and may conceivably be due to a high concentration of chorionic gonadotropin or possibly to an excess of insulin from the hyperplastic fetal islets of Langerhans.

(7) *Placenta*—In this series many of the placentas seemed to be definitely heavier than normal. Whether this is related to maturity remains to be seen. No changes considered to be significant were found in the ad-

and spleen hyperplasia of islets of Langerhans and hematopoiesis) has been noted and provides interesting opportunity for speculation. If it could be shown that anoxia stimulates the islets to hyperplasia, a possible common ground for the two conditions might be found. As yet no explanation for the similarity is at hand. However as noted by Miller<sup>10, 11</sup> caution must be exercised to avoid confusing the two especially when diag-

nosis is based on pathological examination alone in the absence of adequate information concerning the mother

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## Chapter 24

### CANCER AND DIABETES

A RELATIONSHIP between cancer and diabetes has long been suspected but has been difficult to establish on sound statistical grounds. Such an association was mentioned but not elaborated upon by Wilson and Maher.<sup>1</sup> Marble<sup>2</sup> analyzed 256 cases in which the two diseases were associated. These represented the cases of cancer recognized among 10,000 cases of diabetes, i.e. an incidence of 2.56 per cent. Marble also studied the figures of insurance companies and other data and concluded that while certain evidence suggested that cancer was more common among diabetics, such a conclusion could be questioned.

Dry and Lessner<sup>3</sup> reviewed 182 cases of diabetes and found 38 cases of cancer, a remarkably high incidence of 20.9 per cent. It is of interest that 16 of these 38 cases had carcinoma of the pancreas.

Illinger and Landsman<sup>4</sup> in studying 1,280 cases of diabetes found 39 cases of cancer, i.e. an incidence of 3.04 per cent. This they found to correlate well with an incidence of 2.95 per cent in 14,332 cases of diabetes from the world's literature. They compared these figures with an incidence of cancer for the general population of 11.46 per cent (figures for New York State in 1941).

Apparently the most careful statistical study of the problem to date is that of Jacobson.<sup>5</sup> He notes the importance of avoiding Wilson's fallacy of operational selection and of studying the universe of the living as well as the universe of the dead. He also points out that it is not sufficient to show that a given condition is less frequently associated with another at the time of death; rather it must be shown that the first condition is less frequently associated with the second than it is with other diseases and conditions. He demonstrates that cancer is as frequently associated with diabetes as it is with other conditions (e.g. syphilis, tuberculosis) that are generally thought to be associated with cancer and that cancer is more frequently associated with diabetes than it is with other conditions (e.g. cardiovascular renal disease) that are not thought to be associated with cancer. Jacobson concludes, applying Wilson's principle that cancer and diabetes were reported as joint causes of death in New Jersey in 1940 and in New York City in 1930 more frequently than would be expected if these two causes fell independently upon the total population. His general conclusion is that the incidence of cancer is higher among diabetics than among nondiabetic individuals.

The incidence of cancer as a cause of death in the present series was 10 per cent (81 of 818 cases—see Appendix C). This figure is doubtless in part attributable to the fact that the New England Deaconess Hospital is recognized as a center for the treatment of cancer as well as diabetes. Of some interest is the distribution of types of cancer according to tissue and organ systems in diabetes. In Table 45 are presented the sites of origin of 132 cases of cancer occurring in diabetics. Although few conclusions



Fig. 110 — Adenomatous post partum hyperplasia of endometrium in diabetic woman treated during pregnancy with estrogens ( $\times 12$ ).

can be drawn from such a small group it seems apparent that the incidence of carcinoma of the endometrium, carcinoma of the pancreas and pheochromocytoma of the adrenal is higher than would be expected in a group of nondiabetics of similar size.

In a series of patients with carcinoma of the endometrium Moss<sup>7</sup> noticed a rather high incidence of diabetes. He thought that this was probably to be expected in view of the obesity and age of patients suffering from this type of cancer. Messner and Summers<sup>8</sup> have studied the post-partum endometrial hyperplasia found in some diabetic women who were

curetted from three months to two years after delivery. These women had been maintained during pregnancy on Dr. Pri cilla White's regimen of large doses of stilbestrol and progesterone and these hormones were presumably responsible at least in part for the findings which consisted of endometrial hyperplasia, often polypoid, sometimes cystic, sometimes adenomatous, sometimes a combination of both.\* This hyperplasia differed from the ordinary type in involving a more regular and more florid overgrowth of both glands and stroma and in being quantitatively more marked. In typical cases the glands were closely packed, tortuous, adenomatous and dilated with abundant compact cellular stroma often containing eosinophils (Fig. 110). The authors point out that in some series of endometrial carcinoma from 9 to 11 per cent of the patients are diabetics.<sup>7, 10</sup>

Of interest in this connection is a study by Sommers<sup>10</sup> and yet incomplete of a series of multiple malignancies in which a small group of women has been found showing various morphologic stigmata of hyperpituitarism. These women tend to have diabetes mellitus and have multiple cancers involving the endometrium, ovaries, breast and gastro-intestinal tract in various combinations.

Of considerable interest is the 14 per cent incidence of carcinoma of the pancreas in Table 45. This stands in contrast to figures of from 2.5 to 4.8 per cent for the incidence of carcinoma of the pancreas in the general population.<sup>1</sup> The question of a possible etiological relationship at once arises and with this in view the 19 cases are listed in Table 46 in an attempt to compare the duration of the cancer and of the diabetes. It is evident that in over half the cases the symptoms of carcinoma either antedated the symptoms of diabetes or occurred apparently simultaneously with them. This time relation does not of course necessarily indicate a causal relation but certainly makes it more likely.

In this connection it may be noted that Dishell and Palmer<sup>1</sup> in a series of cases of carcinoma of the pancreas found glycosuria (usually inconstant) in 27 per cent and a diabetic type of glucose tolerance curve in 18 of 21 patients. They suggest that the value of this test in the diagnosis of carcinoma of the pancreas may be greater than has been realized. Levy and Lichtman<sup>2</sup> made similar observations. However as noted by Marble<sup>3</sup> cancer patients of any type are poor subjects for the glucose tolerance test and it would seem that all such observations should be carefully controlled.

Silver and Lubliner<sup>4</sup> found that 14 of 43 cases of carcinoma of the body or tail of the pancreas had diabetes, while only 2 of 57 cases of carcinoma of the head of the pancreas had diabetes. One might of course suspect that this was true because carcinoma of the head of the organ is likely to produce biliary obstruction and death before enough of the pancreas is destroyed to bring out the diabetic syndrome.

\* In a more recent series of pregnant diabetic women the complication of carcinoma of the endometrium is less frequent because of the use of a different schedule for administration of the hormones.<sup>11</sup>

TABLE 45 —CANCER COMPLICATING DIABETES

	Male	Female	Total
Breast	0	12	12
Lung	1	2	3
Alimentary tract	0	1	1
Lip	1	0	1
Buccal mucosa	1	0	1
Pharynx	1	0	1
Tongue	1	0	1
Stomach	1	1	2
Colon	8	8	16
Rectum	8	2	10
Pancreas	12	7	19
Liver	1	0	1
Gall bladder and bile ducts	0	1	1
Adrenals (pheochromocytoma)	3	0	3
Kidney	1	2	3
Bladder	4	2	6
Prostate	12	0	12
Uterus (endometrium)	0	4	4
Cervix	0	2	2
Vagina	0	1	1
Ovary	0	2	2
Leukemia	1	2	3
Malignant lymphoma (Hodgkin's)	1	1	2
Thyroid	1	2	3
Skin	2	2	4
Miscellaneous	1	2	3
	—	—	—
Total	67	61	128

TABLE 46 —DURATION OF DIABETES IN RELATION TO CARCINOMA OF THE PANCREAS

Patk. No.	Duration of diabetes	Duration of carcinoma	
DA 267	3 3 yr	1 2 yr	* Simultaneous
55384	1 mo. at out	About 4 mo.	Simultaneous
10840	1 2 yr	1 yr	
12480	5 mo.	Less than 1 yr (1 2 mo.)	Simultaneous
13372	10 yr	3 yr	
16295	6 mo.	1 yr	Antedates
19061	1 5 yr	1 yr	* Simultaneous
22481	3 mo.	Less than 1 yr (1 2 mo.)	* Simultaneous
29273	10 2 yr	1 yr	
32832	4 yr	Not stated	
57232	5 wk.	1 mo.	Simultaneous
60311	1 mo.	1 yr	Antedates
68600	8 yr	8 mo.	
74611	12 yr	Less than 1 yr	
81320	7 5 yr	Not stated	
82595	21 yr	Not stated	
84772	* 6 wk.	Discovered at admission (1 wk.)	* Simultaneous
86702	4 yr	3 yr	* Simultaneous
102682	13 mo.	1 yr 2 wk.	Simultaneous



The strikingly high incidence of primary carcinoma of the liver in hemochromatosis is unexplained. The subject is reviewed by Warren and Drake' (*see also* Chapter 7).

The important subject of tumors of the endocrine glands in relation to diabetes is discussed elsewhere (*see especially* Chapter 16).

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## Chapter 25

### EXPERIMENTAL DIABETES

In the following discussion emphasis will be placed on the pathogenesis and histopathology of experimental diabetes.<sup>1</sup> For further details and more complete bibliography, several excellent reviews may be consulted especially those of Lukens<sup>1,2</sup> Houssay<sup>3,4,5</sup> Buley,<sup>6</sup> Duff,<sup>7</sup> Bell,<sup>8</sup> Haist,<sup>9</sup> Lazarow,<sup>10</sup> Young,<sup>11</sup> Bennett and Evans<sup>12</sup> and Jores.<sup>13</sup> None of these by itself constitutes a complete survey of the field but all taken together provide fairly adequate coverage of the various subdivisions.

In the light of present knowledge it may be stated that all types of permanent experimental diabetes are all types in which the diabetic state is irreversible and persists after discontinuance of the exciting agent depend upon destruction of the  $\beta$  cells of the islets of Langerhans in the pancreas. In all cases therefore there is insufficiency of the endocrine part of the pancreas which is incapable of secreting the amount of insulin required by the organism. Even when the diabetes is initiated by extra pancreatic factors (as e.g. in pituitary diabetes) the development of a permanent diabetic state is accompanied by and dependent upon demonstrable lesions of the  $\beta$  cells of the islets. The pancreas is of course not the only organ which takes part—the presence of the liver for instance is essential for the production of a diabetic state—damage to the pancreas is however the *sine qua non*.

Numerous methods are available for producing a temporary glycosuria with or without hyperglycemia e.g. alimentary by stuffing the organism with excess carbohydrate various drugs such as epinephrine hormones such as some of the adrenal cortical steroids interference with the nervous system as in the *pique* experiment of Claude Bernard and phlorhizin which has the peculiar effect of rendering the kidney tubules incapable of reabsorbing sugar thereby leading eventually to increased ketogenesis and lowered R.Q. Some of these types of temporary diabetes will be mentioned again at the end of the chapter.

There are five principal methods for producing a permanent diabetes.

- 1) Total or subtotal pancreatectomy
- 2) Injection of alloxan or similar substances (including dehydroascorbic acid)
- 3) Administration of anterior pituitary extract
- 4) Thyroid feeding in the partly depancreatized animal
- 5) Administration of excessive amounts of glucose over a prolonged period.\*

\* At the present writing the diabetic state produced by ACTH or a adrenal cortical steroid is just not beyond what is permanent. It is therefore omitted from this list and discussed at the end of the chapter.

**Pancreatectomy**—Ever since the classical experiments of von Mering and Minkowski<sup>18</sup> it has been recognized that total removal of the pancreas results in diabetes. Since total pancreatectomy also leads to various undesirable side effects subtotal pancreatectomy has been more extensively used. Removal of the greater part of the pancreas (nine-tenths in the dog five-sixths in the cat 95 per cent in the rat) usually leads to a mild diabetes which may become permanent. In the dog subtotal pancreatectomy does not produce diabetes if as much as one-seventh to one-eighth of the pancreas is left in provided that insulin treatment is given during the first week after operation and that aseptic technique is followed. When a larger amount of the gland is removed there frequently occurs a mild diabetes (Sandmeyer's diabetes) which is not stable may either disappear spontaneously or increase in severity and lead to death of the animal. This mild diabetes (and in fact any type of experimental diabetes)<sup>19</sup> is made worse by high carbohydrate diet in some instances a dog with a subtotal pancreatectomy and a normal blood sugar may be made diabetic by feeding sugar. In any case the diabetes does not remain on a plateau as regards severity it either disappears or gets worse. An interesting feature of subtotal pancreatectomy in the rat is the fact that diabetes does not appear immediately but only after a lapse of two or three months. Housay<sup>20</sup> points out that this period is equivalent to several years in the life span of man and suggests that a subclinical prediabetic state without symptoms may precede the appearance of human diabetes.

The lesions in the islets after subtotal pancreatectomy were first clearly described in dogs by Allen<sup>21</sup> and in dogs and cats by Homans<sup>18 19 20</sup> aided by the then new staining techniques of Lane and of Bensley. They noted within a few days after the operation diminution in the granules of the  $\beta$  cells accompanied or followed soon by swelling of the cytoplasm and the appearance of clear vacuoles which contained nothing stainable by the methods which are usually used to demonstrate fat glycogen or other substances. For a time the cell membranes remained intact eventually however rupture and disintegration occurred followed finally by disappearance of the  $\beta$  cells. During this process the  $\alpha$  cells preserved their integrity and no leukocytic exudate was observed. The vacuolated appearance of the  $\beta$  cells in the early phase is termed hydropic change and is seen in other types of experimental diabetes. Vacuolation of the epithelial cells of the small ducts of the pancreas may also be seen. (As noted in Chapter 3 the recent work of Toreson<sup>22</sup> indicates that hydropic change in some if not in all instances actually represents glycogen deposition.)

The rapidity of development of these lesions was found by Allen to be dependent on the severity of the diabetes. In severe unchecked diabetes the earliest vacuoles appeared in about five days marked or maximal vacuolation in three weeks and after six weeks to two months all  $\beta$  cells had usually disappeared.

The question of reversibility of the hydropic change is an interesting one. Allen<sup>17</sup> found that the stage of maximal vacuolation usually lasted for about five days. During this period he tried unsuccessfully to abolish the lesions by fasting the animals. Later, however, with the advent of insulin, it was clearly shown by others<sup>22</sup> that reversal of the hydropic change could be achieved by treatment with insulin. This phenomenon becomes more understandable if it is indeed true that the hydropic appearance is due to glycogen.<sup>23</sup>

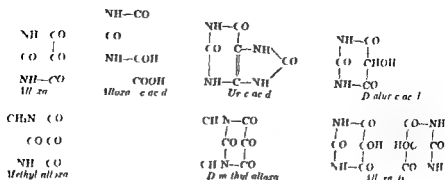
The pathogenesis of the lesions in the islets is a problem to which Allen devoted much attention. He found no evidence that nervous or circulatory factors were concerned. He noted that the severity and rapidity of appearance of the changes varied directly with the severity of the diabetes, that a high carbohydrate diet facilitated their development, and that they could be prevented by fasting the animal or giving a pure fat diet. He found, however, that production of an intense hyperglycemia over a period of three to thirty-eight hours did not produce vacuolation in nondiabetic animals, nor did it lead to perceptible increase in vacuolation in animals already diabetic. Also, in his hands, phlorhizin did not prevent the appearance of hydropic change after pancreatectomy, even though the blood sugar was kept at or below normal levels. Also, phlorhizin glycosuria did not produce vacuolation in nondiabetic animals. Allen therefore concluded that the hydropic change was not dependent on nervous or circulatory factors and not due to hyperglycemia or glycosuria. He postulated a functional overstrain of the islets due to some unknown humoral agent. As will be seen below, more recent evidence suggests that hyperglycemia *per se* (or else some factor or factors constantly associated with hyperglycemia) may be actually due to  
Yet it seems  
this glycogen

deposition with insulin even in the presence of hyperglycemia.<sup>24</sup>

Total pancreatectomy, of course, exerts its effect by sudden and complete removal of the insulin-producing  $\beta$  cells of the islets. Subtotal pancreatectomy removes most of the  $\beta$  cells and may lead to secondary damage of those that survive. It is a fact that the diabetes due to subtotal pancreatectomy (or alloxan) commonly requires for its control more insulin than that due to total pancreatectomy. This finding is discussed in the next chapter.

**Alloxan.** In 1913 Dunn, Sheehan, and McLeitch<sup>25</sup> reported that intravenous injection of alloxan into rabbits produced an initial hyperglycemia followed by hypoglycemia and death accompanied by necrosis of the islets of Langerhans in the pancreas. This announcement was soon followed by the almost simultaneous reports of Brunschwig *et al.*<sup>26</sup> and Bailey and Bailey<sup>27</sup> that permanent diabetes could be produced by this method.

Alloxan is the anhydride of mesoxalic acid. Its structural formula and those of some related compounds are shown in the accompanying diagram.



Of these compounds all are diabetogenic except alloxanic acid and uric acid and dimethyl alloxan. One of the essentials in determining diabetogenic activity appears to be the presence of an intact pyrimidine ring. Activity is abolished by substitution of the alloxan molecule at any position other than in one imino group but even in this site if the substitution is carried beyond a certain length of side chain (e.g. butyl alloxan) the compound becomes inactive. It is probable that the diabetogenic effect of certain compounds related to alloxan e.g. diethylalloxanthin dialuric and methyl dialuric acids depends on their being rapidly oxidized in the blood stream to the corresponding alloxans.<sup>27</sup>

Recently Lukens and Kennedy<sup>28</sup> have shown that a styryl quinoline derivative will produce necrosis of  $\beta$  cells comparable to the effect of alloxan.

Alloxan in proper dosage (which varies in different species) has produced permanent diabetes in the rabbit rat hamster dog cat sheep monkey pigeon and man. Other species e.g. guinea pig tortoise are resistant for reasons unknown.<sup>27</sup> The resistance of the guinea pig may be due to its high blood glutathione.<sup>29</sup>

The typical response of the blood sugar following the intravenous injection of alloxan is represented by a triphasic curve: (1) a phase of hyperglycemia of unknown cause appearing two to four hours after injection; (2) a hypoglycemic phase at six to twelve hours possibly due to blockage of glycogenolysis in the liver<sup>30,31</sup> and (3) a permanent hyperglycemia ensuing at eighteen to twenty-four hours. In some animals the resulting diabetes

tests for insulin tolerance or sensitivity resemble those seen in depauperatized animals. The severity of the diabetes may be modified by hypophysectomy,<sup>32</sup> adrenalectomy,<sup>33</sup> thyroidectomy or feeding thyroid extract.<sup>34</sup>

et that it  
on<sup>35</sup> that  
and that

the earliest response curve

clamping the pancreatic vessels for five minutes during and after the injection prevents the necrosis of the islets<sup>28</sup>. Clamping the renal or splenic pedicle has also a preventive effect perhaps due to a vasomotor reflex affecting the pancreas<sup>29</sup>. The action of alloxan may also be prevented by the injection of various substances *e.g.*, glutathione, cysteine, H<sub>2</sub>AL<sub>2</sub>, and many others provided that they are injected either immediately before simultaneously with or within a few minutes after the injection of alloxan<sup>2, 10, 21, 22</sup>. One of the interesting side effects of alloxan is the marked fall in blood glutathione which follows its injection<sup>24</sup>. Alloxan added to plasma is converted almost entirely to alloxanic acid<sup>40</sup>. This stable conversion product may provide a valuable means of studying the relation if any of alloxan to human diabetes.

The histopathology of the islets in alloxan diabetes has been studied most carefully in rabbits, rats and dogs and has been particularly well described by Bailey, Bailey and Hagan<sup>25</sup> and by Hughes, Ware and Young<sup>4</sup>. The earliest changes are discernible as early as five minutes after injection of a diabetogenic dose of alloxan and consist in a slight diminution in number of granules in the  $\beta$  cells with slightly increased prominence of the nuclear membrane and some irregularity of the cell outline. After ten to fifteen minutes there is a definite reduction of granules affecting first the  $\beta$  cells in the centers of the larger islets. At one hour there is evidence

appearance with homogeneous eosinophilic cytoplasm. Thereafter disintegration of both cytoplasm and nuclei becomes more apparent with coalescence of cells and evidence of karyolysis. By the end of twenty-four hours extensive disintegration and disappearance of cells is the rule; the centers of the islets usually containing only amorphous granular debris. Only after about forty-eight hours<sup>25</sup> there is a marked fall in insulin content of the pancreas<sup>41</sup>. After several days the granular material is removed by an unknown mechanism and the islet may consist only of a peripheral layer of  $\alpha$  cells. The latter cells are spared according to most reports although some writers describe complete disappearance of islets and some report increase in number of  $\alpha$  cells. Eventually one may find shrunken islets made up only of these cells (Plate III). In other instances a few  $\beta$  cells may survive. The fate of the D cells in alloxan diabetes is not known with certainty, probably because most authors have not used the specialized and rather difficult techniques necessary to demonstrate them. (See also p. 24).

Throughout the entire sequence there is a striking absence of the leukocytic infiltration usually associated with necrosis of tissue in other well-recognized pathological processes. Also the lack of eventual fibrosis or hyalinization of the islets is an interesting feature although this may per-

haps be explained as due to the rather specific action of alloxan on the  $\beta$  cells without associated damage to the connective tissue stroma.

Hydropic change of the  $\beta$  cells which is so characteristic of other types of experimental diabetes as noted elsewhere in this chapter is a late phenomenon in alloxan diabetes. It does not appear in the early sequence (described above) which follows the injection of a single diabetogenic dose of alloxan probably because the injury to the cells is so acute and severe. Occasionally hydropic change may be observed after several weeks in animals made diabetic in this way and has been attributed by Kennedy and Lukens<sup>44</sup> to the effect of hyperglycemia on  $\beta$  cells which escaped destruction by the alloxan. Bailey *et al.*<sup>45</sup> noted hydropic change and mitotic figures in some of the islets of animals given repeated small doses of alloxan. Töreson<sup>41</sup> has demonstrated the presence of glycogen in the hydropic  $\beta$  cells in alloxan diabetes and Duff and Töreson<sup>42</sup> have made the remarkable observation that the deposition of glycogen in the islands can be prevented or reversed by small doses of insulin even in the presence of hyperglycemia. They conclude that hypoinsulinemia may be more important than hyperglycemia in producing this change.

Goldner and Gomori<sup>46</sup> described marked hydropic change in epithelium of the intralobular ducts of dogs made diabetic with alloxan and examined after sixteen to eighteen days. Duff and Starr<sup>47</sup> observed mitotic figures reaching a peak at seventeen hours in the acinar tissue of hooded rats. Usually the acini of the pancreas show no change. In animals which recover from transient diabetes induced by a single dose of alloxan there is apparently active regeneration of  $\beta$  cells<sup>47</sup> reaching a peak on the fourth day as indicated by colchicine<sup>48</sup>. This regenerative activity appears to be increased by the administration of anterior pituitary extract<sup>49, 50</sup>.

Ruangsuri<sup>51</sup> reported observations of the acute effect of alloxan on the living pancreas treated with neutral red and Janus green. He described clefts of fluid in the  $\beta$  cells in less than thirty minutes after injection of the drug. These clefts subsequently enlarged and fluid escaped into the intercellular spaces at the same time that the cytoplasmic granules were disappearing. Later fragmentation of nuclei was seen. Attempts to confirm these observations in this laboratory have been unsuccessful because of inability to achieve adequate visualization of the  $\beta$  cells *in vivo*.

The kidney is perhaps next in importance to the pancreas as a site of lesions in alloxan diabetes. The severest damage occurs in the convoluted tubules and appears to be generally proportional to the size of the dose. The epithelium of the tubules may show changes varying from slight swelling and vacuolation to severe necrosis and desquamation leading at times to death of the animal in uremia. These findings are apparently limited to the convoluted tubules and the renal lesions usually disappear with the uremia. The nature of the lesions in the tubules may be found if the terminal blood sugar is normal.

or higher<sup>42</sup> Inter-capillary glomerulo-sclerosis has not been reported. However, Mann and Goddard<sup>43</sup> have found a somewhat similar change, consisting of thickening of the basement membrane deposition of a hyaline material between the capillaries and unlike the human lesion, glomerular adhesions and proliferation of endothelial and epithelial cells. (See Chapter 12)

Lesions of the liver are apparently unimportant in animals given ordinary doses of alloxan although focal necrosis is described. Dogs given large doses may exhibit jaundice and other evidence of hepatic damage. In dogs which have been diabetic for some weeks fatty change in the liver may be observed but since fatty livers are also observed in depancreatized dogs this observation is of questionable significance in relation to the direct effects of alloxan. Necrosis observed occasionally in dogs cats and rats is more probably due to the drug. Herbut *et al*<sup>44</sup> have described severe necrosis leading to portal cirrhosis with deposition of iron resembling hemochromatosis in rabbits given a high cholesterol diet following the injection of alloxan. Liver glycogen is said to be normal or even increased in alloxan diabetes<sup>45</sup> but this may vary depending on the severity of the diabetes.

In the adrenal glands focal necrosis of both cortex and medulla has been reported. The thyroid may be somewhat atrophic. Lesions of the eyes include cataracts similar to those of man. Necrosis of the basophil cells of the pituitary may follow massive doses of alloxan. Arterio-sclerosis of severe degree has not been reported in fact alloxan diabetes seems actually to inhibit the development of cholesterol athero-sclerosis in the rabbit<sup>46</sup> (see also Chapter 9). Alloxan diabetes has caused dwarfing of young rats and abortion and stillbirths have occurred in a large percentage of pregnant diabetic rabbits.<sup>47</sup>

It is usually stated that the human islets are resistant to the action of alloxan. This opinion as Lukens<sup>2</sup> points out seems to be based chiefly on the work of Brunschwig *et al*<sup>48</sup> who gave alloxan in varying doses to a patient with islet cell carcinoma and to four other patients with advanced malignant disease of other types without producing diabetes or characteristic lesions of the islets at necropsy. These investigators however gave the drug in large amounts of saline and therefore slowly. In view of the known rapidity with which alloxan is destroyed in the blood stream the doses which they used can hardly be considered comparable to those successfully employed in the experimental animal. In two other cases<sup>49,50</sup> definite relief of hypoglycemia has followed the use of alloxan and in one case reported by Conn *et al*<sup>50,51</sup> although an islet cell tumor was found to be resistant the islets in the remainder of the patient's pancreas were damaged and permanent diabetes ensued. This patient received a total of 800 mg. of alloxan per kg. over a period of nine days. When the tumor was removed the  $\beta$  cells of which it was largely composed were found to be apparently intact while the  $\beta$  cells of the islets proper showed varying de-



gress of degeneration from dehydration and hydropic change to loss of cell membranes, pyknosis and fragmentation of nuclei and complete disappearance of cells. These authors conclude that the sensitivity of the  $\beta$  cells of man is comparable to that of susceptible animals and that alloxan should not be used as a therapeutic agent.

It seems clear that the diabetes which follows injection of alloxan is due essentially to partial pancreatectomy induced by chemical means. However the exact mechanism of action of the drug on the islets and the cause of its remarkable specificity for the  $\beta$  cells are unknown.<sup>27, 28</sup> As noted above the effect of alloxan occurs within five minutes after intravenous injection as shown by its rapid disappearance from the blood and by the prevention of its action by clamping the pancreatic vessels. Its effect is clearly not due to the triphasic blood sugar curve described above since prevention of the initial hyperglycemic phase (by insulin phlorizin or other means) does not prevent the lesions of the islets or the ensuing

primary ligation of the pancreatic duct for reasons which are not clear.<sup>29, 30</sup>

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Of great interest is the recent production of diabetes with dehydroascorbic acid, a substance which occurs naturally in the body and which is similar in structure and chemical properties to alloxan. This diabetes can also be prevented with glutathione and related compounds.<sup>33, 34</sup>

On the supposition that alloxan may act by combining with zinc Endota<sup>35</sup> has tried other organic substances reacting with zinc and has claimed successful production of diabetes in rabbits with oxine and dithione. The histologic changes in the islets are said to be similar to those produced by alloxan.

**Pituitary Diabetes**—In 1927 John O Mulsomm, Potts and Laughton<sup>36</sup> described a temporary diabetes following the injection of an extract of the anterior lobe of the pituitary in dogs. This report was followed in 1932 by papers from three different groups of investigators on the production of

pituitary diabetes<sup>10,11</sup> Finally in 1937 Young<sup>12</sup> made the important observation that daily injections in dogs of crude extracts of anterior pituitary over a period of weeks would lead to a permanent diabetic state which persisted after discontinuance of the injections. Richardson and Young<sup>13</sup> described lesions of the islets in such dogs.

The physiology of pituitary diabetes is complex and has recently been reviewed by Lukens,<sup>1</sup> Young<sup>12</sup> and Bennett and Evans<sup>14</sup> to whose papers the reader is referred for further details. The thyrotropic, lactogenic and gonadotropic factors of the anterior pituitary appear to have no diabetogenic effect and the production of diabetes seems to depend on a combination of the growth and adrenotropic hormones. Recently it has been shown that purified growth hormone at least in some animals is a more potent diabetogenic agent than A.C.T.H.<sup>15,16,17</sup> The mechanism is obscure but seems to involve both suppressed utilization and overproduction of carbohydrate. Inhibition of hexokinase may play a part in the process.<sup>17</sup>

When a dog is given daily injections of a suitable crude aqueous extract of anterior pituitary hyperglycaemia occurs associated with a progressive loss of granulation in the  $\beta$  cells a process which requires about seven days to reach its maximum. At about this time hydropic change (glycogen<sup>18</sup>) appears. The diabetes is now reversible and if injections are stopped recovery may occur and the granules may reappear in the  $\beta$  cells. If however the injections are continued permanent diabetes (called "metahypophyseal diabetes" by Honnig<sup>19</sup> and Young<sup>12</sup>) will usually ensue in two to four weeks. Thereafter discontinuance of the injections does not affect the diabetes and when the pancreas is examined the islets are found to be small and composed almost entirely of  $\alpha$  cells. As in the diabetes following subtotal pancreatectomy hydropic change may be seen in the epithelial cells of the small ducts also and in the early stages mitotic figures may be seen in these cells as well as in the  $\beta$  cells.<sup>20</sup> Hyalinization of the islets has been recorded<sup>19,21</sup> but is exceedingly rare in contrast to the human disease. Interstitial glomerulosclerosis has been described in the kidneys of a dog maintained in the diabetic state for five years.<sup>22</sup>

The ameliorating effect of pituitary extract in alloxan diabetes is observed by Ogilvie<sup>23,24</sup> and Cavallero<sup>25</sup> has not been elucidated. It is perhaps due to a pancreatropic agent (the growth factor?). Many species of animals do not respond with diabetes to the injection of anterior pituitary extract. In young rats and rabbits this treatment leads to an increase in the volume of islet tissue and an increase in insulin content of the pancreas a phenomenon which Marks and Young<sup>26</sup> ascribed to a pancreatropic factor in the extract. In the total injection of pituitary extract or implantation of anterior pituitary has no diabetogenic effect when the pancreas is intact, but leads to marked increase in severity of pancreatic diabetes.<sup>27</sup>

Purified growth hormone is diabetogenic in the mature adult dog and cat but not in the puppy or kitten. In other species its effect is variable.<sup>28</sup> These interesting species differences are probably related to similar differ-

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pituitary diabetes.<sup>68 70 71</sup> Finally in 1937 Young<sup>72</sup> made the important observation that daily injections in dogs of crude extracts of anterior pituitary over a period of weeks would lead to a permanent diabetic state which persisted after discontinuance of the injections. Richardson and Young<sup>73</sup> described lesions of the islets in such dogs.

The physiology of pituitary diabetes is complex and has recently been reviewed by Lukens,<sup>1</sup> Young<sup>74</sup> and Bennett and Evans<sup>75</sup> to whose papers the reader is referred for further details. The thyrotropic, luteogenic and gonadotropic factors of the anterior pituitary appear to have no diabetogenic effect and the production of diabetes seems to depend on a combination of the growth and adrenotropic hormones. Recently it has been shown that purified growth hormone at least in some animals is a more potent diabetogenic agent than ACTH.<sup>76 77 78 79</sup> The mechanism is obscure but seems to involve both suppressed utilization and overproduction of carbohydrate. Inhibition of hexokinase may play a part in the process.<sup>77</sup>

When a dog is given daily injections of a suitable crude aqueous extract of anterior pituitary hyperglycemia occurs associated with a progressive loss of granulation in the  $\beta$  cells, a process which requires about seven days to reach its maximum. At about this time hydropic change (glycogen<sup>80</sup>) appears. The diabetes is now reversible and if injections are stopped recovery may occur and the granules may reappear in the  $\beta$  cells. If however the injections are continued permanent diabetes (called metahypophyseal diabetes by Houssay<sup>8</sup> and Young<sup>12</sup>) will usually ensue in two to four weeks. Thereafter discontinuance of the injections does not affect the diabetes and when the pancreas is examined the islets are found to be small and composed almost entirely of  $\alpha$  cells. As in the diabetes following subtotal pancreatectomy hydropic change may be seen in the epithelial cells of the small ducts also and in the early stages mitotic figures may be seen in these cells as well as in the  $\beta$  cells.<sup>78</sup> Hyalinization of the islets has been recorded<sup>79 81</sup> but is exceedingly rare in contrast to the human disease. Inter-capillary glomerulosclerosis has been described in the kidneys of a dog maintained in the diabetic state for five years.<sup>80</sup>

The ameliorating effect of pituitary extract in alloxan diabetes is observed by Ogilvie<sup>48 60</sup> and Cavillero<sup>49</sup> has not been elucidated. It is perhaps due to a pancreatropic agent (the growth factor<sup>24</sup>). Many species of animals do not respond with diabetes to the injection of anterior pituitary extract. In young rats and rabbits this treatment leads to an increase in the volume of islet tissue and an increase in insulin content of the pancreas, a phenomenon which Marks and Young<sup>8</sup> ascribed to a pancreatropic factor in the extract. In the adult injection of pituitary extract or implantation of anterior pituitary has no diabetogenic effect when the pancreas is intact but leads to marked increase in severity of pancreatic diabetes.<sup>8</sup>

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When a dog is given daily injections of a suitable crude aqueous extract of anterior pituitary hyperglycemia occurs associated with a progressive loss of granulation in the  $\beta$  cells, a process which requires about seven days to reach its maximum. At about this time hydropic change (glycogen<sup>60</sup>) appears. The diabetes is now reversible and if injections are stopped rec

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ences in the response to partial pancreatectomy which in turn may perhaps be correlated with the habits and environment of the animals.<sup>11, 12</sup>

Lukens and Dohan<sup>16, 17</sup> were able to produce diabetes in cats in which one half to three fourths of the pancreas had been previously removed. The pathological changes were similar to those in the dog but the phase of hydropic change persisted for about three months before the cells disintegrated and the diabetes became irreversible. There is thus a considerable period in case of the cat in which procedures designed to cure the diabetes may be tried. During this three months Lukens and Dohan<sup>17</sup> were able to cause disappearance of diabetic symptoms and restoration of the islets to practically a normal state by means of (1) insulin (2) phlorizin (3) diet and (4) adrenalectomy. After the permanent phase of atrophy of the islets had supervened treatment was of no avail.

The pathology and pathogenesis of pituitary diabetes have been carefully studied by Ham and Haist.<sup>18</sup> These authors noted the resemblance of the lesions to those described by Allen<sup>17</sup> following subtotal pancreatectomy and like him postulated excessive function due to some unknown stimulus as the effective cause of the degranulation, hydropic change and final disappearance of the  $\beta$  cells. Ham and Haist suggest that pituitary extract may increase the need for insulin by promoting overproduction and under

are involved in the sequence—an early phase, due to increased need for insulin and the late permanent phase due to decreased production of insulin (these would correspond to the idio- and metahypophyseal types). Diabetes could thus be considered in either case as due to increase of the ratio of insulin need to insulin production.

Lukens and his co-workers have submitted evidence that hyperglycemia may be the effective stimulus to the  $\beta$  cells. They find that the lesions of the  $\beta$  cells can be prevented if the blood sugar is kept at normal levels and that the hydropic change can be reversed by procedures that reduce the

that since both can be used to cure pituitary diabetes at the early lesions in the early stages it is likely that the level of the blood sugar is a major factor in damaging the islets. And yet as Lukens<sup>16</sup> points out in a more recent paper if this be true it is not clear why more cases of human diabetes do not increase in severity nor why the metahypophyseal diabetes of dogs does not get worse. Furthermore as noted above, Duff and Torson<sup>22</sup> have shown that insulin will reverse hydropic change in alloxan dia

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hyperthyroidism and may follow thyroid feeding in animals permanent diabetes has not been produced by this method in intact animals. However when the resistance of the pancreas has been reduced by partial pancreatectomy administration of alloxan<sup>24</sup> or anterior pituitary extract the feeding of thyroid will then either intensify a pre-existing diabetes or induce the diabetic state. If the pancreatic tissue is reduced in an animal by removal of 6/7 or 7/8 of its mass insufficient in itself to produce diabetes the animal is sensitized to thyroid treatment which may then lead to hyperglycemia glycosuria and ketonuria. As treatment is continued lesions of the  $\beta$  cells of the islets may appear consisting of loss of granules hydropic change pyknosis and cytolysis. In the early stages as in some other forms of experimental diabetes these lesions are reversible and cessation of thyroid feeding leads to recovery of the animal. This stage is called by Houssay thyroid diabetes. If however the feeding of thyroid is continued over a period of several weeks the lesions may become irreversible and the diabetes may remain permanently even though no more thyroid be given. This permanent irreversible phase Houssay terms metathyroid diabetes. It is characterized by complete degeneration of  $\beta$  cells. This phase of the diabetes becomes progressively worse presumably due to the continuing gradual destruction of the  $\beta$  cells.

The mechanisms involved in the first or extrapancreatic phase of thyroid diabetes are obscure but may be related to the ability of thyroid extract to increase intestinal absorption of glucose and to decrease liver glycogen. Later in the metathyroid stage a permanent pancreatic diabetes ensues (as in pituitary diabetes) presumably due to the injurious effect on the  $\beta$  cells of the long-continued hyperglycemia or of fact is intimately associated with the hyperglycemia.

**Administration of Glucose**—The constant association of hyperglycemia with lesions of the  $\beta$  cells produced by any means other than alloxan has been noted above. Recently Dohan and Lukens<sup>25, 26</sup> have succeeded in producing hydropic change of the  $\beta$  cells and permanent diabetes in rats solely by administration of glucose. Although this work is as yet unconfirmed its implications are of such importance that it is included here as a fourth method for the production of permanent diabetes. These authors using both normal and partially pancreatectomized rats were able by means of repeated intraperitoneal injection of 20 per cent glucose in saline to produce sustained hyperglycemia and hydropic change of the  $\beta$  cells. They report animals (both normal and partially depancreatized) maintained in hyperglycemia for periods varying from several days to several weeks. The development of islet lesions was related to the degree and particularly to the duration of the hyperglycemia. One normal animal was kept in a hyperglycemic state for thirty nine days and remained severely diabetic until twenty two days after the last injection when it was sacrificed while in diabetic acidosis.

The question of whether the elevated blood sugar stimulates the  $\beta$  cell to the point of functional exhaustion or whether some other mechanism is involved remains to be answered. The authors point out that after the development of hyperglycemia this type of diabetes is quite similar in its course to pituitary diabetes. However in the initial stages there are certain differences as follows. In the first three or four days before lesions of the islands appear crude anterior pituitary extract lowers the RQ inhibits the action of insulin on the blood sugar promotes ketogenesis and thyroid and adrenal hyperplasia occur. There is probably a direct inhibition of the secretion of insulin and the insulin content of the pancreas is reduced. In contrast the administration of glucose initially raises the RQ increases the efficiency of injected insulin is antiketogenic and is not accompanied by thyroid or adrenal hyperplasia. Furthermore glucose stimulates insulin secretion and carbohydrate diet maintains a high insulin content of the pancreas.<sup>10</sup> (See also the more recent discussion of hyperglycemia by Lukens<sup>11</sup>)

### METHODS OF PRODUCING TEMPORARY DIABETES

There are of course numerous methods for producing a temporary elevation of the blood sugar (e.g. various drugs and hormones the piguer experiment of Claude Bernard). Four of these seem of sufficient theoretical interest to be mentioned briefly.

**Diabetogenic Activity of the Adrenal Cortex** — A possible diabetogenic effect of the adrenal cortex was long suspected because (a) adrenalectomy diminishes the severity of pancreatic diabetes in certain animals (b) conversely administration of adrenal cortical extract to animals having a pancreatic diabetes previously attenuated by adrenalectomy or hypophysectomy increases the severity of symptoms and (c) clinical diabetes is often associated with overactivity of the adrenal cortex as e.g. in Cushing's syndrome and the Achard Thiers syndrome or in association with adrenal cortical tumors.<sup>12-14</sup>

When purified adrenal cortical hormones and the adrenotropic hormones of the pituitary became available a considerable stimulus was given to attempts at producing experimental diabetes with these substances. The C-11-oxygenated adrenal cortical steroids seem to act by stimulating gluconeogenesis from protein and probably also by inhibiting the utilization of carbohydrate. In an effort to determine the relative importance and possible interaction of these two factors Ingle *et al.*<sup>15</sup> studied the effects of insulin with particular reference to nitrogen excretion in intact animals given adrenal cortical steroids and in depancreatized animals. The results of the study were difficult to interpret due to the high resistance to insulin shown by the animals with adrenal steroid diabetes. The only report of lesions of the islets in this type of experiment is as far as we are aware that of Kobernick and More<sup>16</sup> who found striking hydropic change (glycogen)

in the islets of rabbits given cortisone. We have been able to confirm this observation in the rabbit (*see* Figs. 13, 14 and Plate III) <sup>91</sup>

Of great interest is the recent experimental production of temporary diabetes in human volunteers by the intramuscular administration of purified adrenocorticotrophic hormone for several days <sup>92, 93</sup>. Not only hyperglycemia and glycosuria have been produced but also diabetic glucose tolerance curves have been obtained.

relationship between uric acid and alloxan: the fact that alloxan causes a fall in blood glutathione and the recent experimental work of Griffiths <sup>94, 100</sup> who produced hyperglycemia in rabbits by first lowering the blood glutathione by deficient diet and then giving uric acid intraperitoneally (*see below*).

The mechanisms underlying the metabolic changes produced by adrenocorticotrophic hormone in man are obscure. It seems clear that increased gluconeogenesis from protein does not explain the whole picture: there appears to be a general decrease in the ability of the tissues to dispose of glucose normally. Conn *et al.* <sup>95</sup> suggest the possibility that a heightened intracellular production of uric acid and its intermediaries produced by adrenocorticotrophic hormone together with a fall of the levels of glutathione and perhaps other sulphydryl bearing substances may diminish enzymatic production or release of pancreatic insulin.

**Estrogens**—Apparently estrogens may have a mildly diabetogenic effect in depancreatized animals of certain species <sup>101, 102</sup> but evidence is conflicting <sup>103, 104</sup>.

**Uric Acid Diabetes**—In two recent as yet unconfirmed reports Griffiths <sup>99, 100</sup> describes the production of hyperglycemia lasting four or five days by the injection of uric acid intraperitoneally into rabbits in which the blood glutathione had previously been lowered by a diet deficient in methionine and cystine. The initial blood sugar curve was said to resemble that produced by alloxan. These results if confirmed would be of considerable theoretical interest in view of the lowering of blood glutathione which follows the injection of alloxan, the prevention of the action of alloxan by glutathione and the effects of adrenergic hormone on uric acid and glutathione cited above.

Collins Williams and Bailey <sup>105</sup> were unable to confirm Griffiths' results. The suggestion of Griffiths <sup>100</sup> that this may have been due to their use of an anemic breed of rabbits does not seem entirely convincing. Grunert and Phillips <sup>96</sup> found that uric acid was not diabetogenic in rats on a diet deficient in methionine and sodium. They admit that they were unable to manipulate the diet so as to produce a low glutathione level in both blood and liver (presumably necessary for uric acid to be converted to an alloxan-like substance in the liver) but cite unpublished work of their own

in which uric acid was found to be converted quantitatively to allantoin by the livers of sulfhydryl-deficient rats

**Experimental Obesity**—It has been shown that experimental obesity produced by hypothalamic lesions<sup>106</sup> may be associated with diabetes<sup>107</sup> thus producing a striking parallel with the known importance of obesity in man

**Summary**—As noted at the beginning of this chapter it seems clear that all forms of permanent irreversible experimental diabetes are associated with and dependent upon lesions of the  $\beta$  cells in the islets of Langerhans. The various methods for producing diabetes in the experimental animal may be grouped according to whether the damage to the islets is (a) primary or (b) secondary

In the first group belong total and subtotal pancreatectomy and alloxan diabetes which is essentially chemical pancreatectomy with respect to the  $\beta$  cells. In these cases there is a direct and immediate reduction of the total

In the secondary group may be placed the experimental diabetes produced by anterior pituitary extract thyroid feeding and the administration of excessive amounts of glucose. In these cases the diabetes is at first temporary and reversible and if the exciting agent is discontinued during the early stages symptoms disappear and the animal returns to an apparently normal state. This may be termed the extrapancreatic phase during which the  $\beta$  cells may show the reversible hydropic change (glycogen) due perhaps to the effect of an increased need of the tissues for insulin. If however the administration of the exciting agent is continued for a sufficient length of time a permanent pancreatic phase ensues accompanied by lysis and disappearance of the  $\beta$  cells

With respect to this secondary group it is noteworthy not only that the same type of lesion in the islets is produced by a variety of agents but also that there is one factor common to all and apparently essential namely prolonged hyperglycemia. When this is prevented as by starvation or phlorizin damage to the islets does not occur. Conversely if the blood sugar is elevated by a high carbohydrate diet or other means the lesions of the  $\beta$  cells are accelerated and intensified. It seems probable therefore that either the hyperglycemia itself or some factor constantly associated with it is responsible for the lesions. However the problem is a complex one and hyperglycemia alone may not be the answer.<sup>24</sup> The work of Duff and Torrance<sup>25</sup> cited above in which reversal of hydropic change in alloxan dia-

Also the important question of the role of insulin as a hormone the hyperglycemic-glycogenolytic (H G) factor, is yet to be elucidated<sup>108 109</sup> (see next chapter for further discussion)

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## Chapter 26

### ETIOLOGY AND PATHOGENESIS OF DIABETES

This difficult subject is ably discussed by Marble<sup>1</sup> Long<sup>2</sup> Mirsky<sup>3,4</sup> Soskin<sup>5</sup> Hunsworth<sup>6</sup> Lukens<sup>7,8</sup> Wilder<sup>9</sup> and Thorn and Lorshum<sup>10</sup> Peters<sup>1</sup> wisely cautions against drawing too hasty generalizations from animal experiments regarding the pathogenesis of the disease in man.

However diverse the underlying etiologic factors may be it is probable that diabetes mellitus has one immediate cause insulin deficiency. This deficiency may be actual through lessened production through neutralization of insulin by hypothetical substances by failure of insulin transport from the islands or it may be relative through increased demand or through insufficient facilities for the formation and storage of glycogen. This is succinctly stated by Long<sup>2</sup> as follows: "It is probably correct to say that the continuance of the disorder of metabolism known as diabetes mellitus rests on a disproportion between the requirements of the organism for insulin and the capacity of the islets of Langerhans to meet this demand." This is not to say that diabetes is a disorder of carbohydrate metabolism alone nor does it imply that insulin is the only hormone and the pancreas the only gland involved in the syndrome. The major role played by the liver in regulating the blood sugar and the apparent antagonism between the anterior pituitary and adrenal glands on the one hand and the pancreas on the other are emphasized especially by Soskin.<sup>5</sup>

### THE PANCREAS

The evidence is overwhelming that whatever the distribution of insulin in the tissues whatever the possible sources of supply the islands of the pancreas are of outstanding importance for insulin production. In fact it now seems reasonably clear from the recent work of Wrenschill *et al.*<sup>1</sup> that the extent of granulation of the cytoplasm of the  $\beta$  cells is closely related to the *pancreatic content of insulin suggesting strongly that the  $\beta$  granules actually represent stored insulin.* If this be true it is natural to look first for lesions of the islands and particularly of the  $\beta$  cells in diabetes.

At one end of the scale we have the relatively easily understood cases of diabetes developing after extensive destruction of pancreatic tissue either suddenly (and rarely) as in acute pancreatitis or gradually as in hemochromatosis. At the other end we have the cases so difficult of explanation in which the various organs are apparently normal although the diabetes is severe. However even here insulin deficiency is demon-

strated by the abnormal glycogen distribution and by the therapeutic effect of insulin

Further evidence for the importance of the pancreas in most cases of human diabetes is the fact that the diabetic pancreas usually contains far less insulin than normal and also that diabetics usually excrete less insulin in their urine although this could be due to an increased rate of destruction. Also it should be stated again with emphasis that in all forms of permanent experimental diabetes without exception lesions of the islets of Langerhans are demonstrable (see Chapter 29).

Anatomical causes of insulin deficiency may be listed as follows

- I Destruction of pancreatic tissue (total in ulcer and cancer)
  - a) Acute
  - b) Chronic (with or without calculus)
- II Malignant disease
  - a) Primary
  - b) Metastatic
- III Hemochromatosis
- IV Toxic injury
  - 1 Surgical removal
  - 2 Selective destruction of isletular tissue
    - a) Amyloid infiltration
    - b) Fibrosis
    - c) Toxic injury with hypoplasia
    - d) Hydropic change (glycogen infiltration)
    - e) Reduction in  $\beta$  cells due to unknown causes
- V Inadequate isletular tissue (congenital deficiencies)
- VI Inadequate blood supply
  - 1 Arteriosclerosis

Most of these lesions have been discussed in Chapter 3 and need not be considered in detail here.

The effect of total pancreaticectomy in man is of great theoretical interest as several well attested cases which survived for a reasonable period are now available and it seems established without question that total pancreaticectomy in man produces a diabetes requiring about 40 units of insulin daily for control. This finding is contrary to expectations since it was formerly thought that total diabetes (*i.e.* that associated with complete loss of isletular tissue) would require large amounts of insulin (about 200 units per day) on the basis of animal experiments. The fact that it does not invites speculation as to the importance in the usually encountered severe case of diabetes of (a) the external secretion of the pancreas (b) contra-insulin factors or hormones (c) peripheral destruction or inactivation of insulin (d) greatly increased metabolic processes requiring large amounts of insulin and (e) the possible effect of the still hypothetical hyperglycemic-glycogenolytic hormone of the  $\alpha$  cells. With regard to (a) the obvious factor of malnutrition in the animal or man totally deprived of his pancreas is not to be neglected. It should be emphasized also

that insulin requirement is not the only nor necessarily the most valid criterion of the severity of diabetes (See further discussion on page 296)

The mechanism by which selective destruction of isular tissue takes place is obscure. Hydropic change (glycogen infiltration<sup>18</sup>) of the  $\beta$  cells is the characteristic lesion of experimental diabetes, no matter how produced, and seems to be associated with if not caused by hyperglycemia, especially in view of the fact that the administration of excessive amounts of glucose alone will produce the lesion (however the effect of hyperglycemia cannot be reduced to a simple formula (see below)). The emphasis of Mirsky<sup>24</sup> and others on the rarity of this lesion in human diabetes would seem to have little relevance since obviously experimental conditions do not obtain and the stimulus whatever it may be probably acts over a period of years rather than days or weeks. Furthermore it is rare that a fresh newly discovered case of diabetes (i.e. the type in which one would expect hydropic change) comes to autopsy. Also if it be true as the work of Duff and Torison<sup>19</sup> suggests that insulin can reverse this change even in the presence of hyperglycemia then one would expect the lesion to be rare in treated patients.

Speculation concerning possible causes for the selective destruction of  $\beta$  cells in the islands (see Chapter 3 for evidence that  $\beta$  cells are reduced in number in many cases of diabetes) was greatly stimulated by the finding that alloxan in proper doses causes such destruction (see Chapter 25). The possible role of alloxan or a closely related substance in the etiology of diabetes has been elaborated particularly by Lazrow.<sup>20,21</sup> This author

observations he suggests that the specialization of the  $\beta$  cells for insulin synthesis may result in a localized depletion of glutathione in these cells. He points out that insulin is 12 per cent cystine and depends on the S-S group for its activity and that the cystine can be formed by oxidation of cysteine which is also a constituent of glutathione. Because of this situation there may be competition for the cysteine reaching the  $\beta$  cells since it is used for synthesis of both insulin and glutathione. As a consequence of this competition Lazrow suggests there may be less cystine available in the  $\beta$  cells for glutathione synthesis than is the case with other cells. It has been further calculated that if the cysteine contained in glutathione

is summed up these hypotheses in a diagram (Figure 25-1).

Houssay<sup>22</sup> has recently published a detailed and well-documented survey of the relation of sulfur compounds to carbohydrate metabolism with particular reference to glutathione, the SH-containing enzymes and the effects of alloxan.

Heightened interest in the possible rôle of alloxan (or a closely related compound) was also provided by the experimental work of Griffiths<sup>25,26</sup> who has claimed to produce diabetes in rabbits with uric acid after preliminary depletion of the blood glutathione level by means of a diet deficient in cystine and methionine but containing large amounts of ascorbic acid (See also Chapter 25)

Cohn<sup>27,28</sup> on the basis of his spectacular work in producing a temporary

### METABOLISM OF BETA CELL

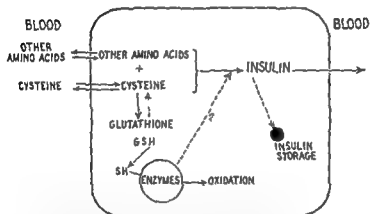


FIG. 111 — Metabolism of the  $\beta$  cell according to Lazarow. (Reproduced by courtesy of Dr. Arnold Lazarow from *Physiological Reviews* 29: 48, 1949.)

Amino-acids, including cystine, enter the  $\beta$  cell from the blood. There, cystine is

are inactivated by alloxan the cells may not only lose their capacity to synthesize insulin but the cells themselves may die because they are unable to carry on their metabolic functions.

According to some investigators<sup>29,30</sup> no deviation from the normal has been demonstrated in the uric acid and glutathione levels in the blood of diabetic patients. However, Housley<sup>31</sup> cites several European writers who found a decrease.

Leech and Marble<sup>32</sup> have studied the total hydrolyzable cystine in the blood serum of 52 diabetics and 25 normal patients. The diabetics showed a trend toward a decreased total cystine in the serum as a whole and in the

that insulin requirement is not the only nor necessarily the most valid criterion of the severity of diabetes (See further discussion on page 296)

The mechanism by which selective destruction of insular tissue takes place is obscure. *Hydropic change* (glycogen infiltration<sup>10</sup>) of the  $\beta$  cells is the characteristic lesion of experimental diabetes no matter how produced and seems to be associated with if not caused by hyperglycemia especially in view of the fact that the administration of excessive amounts of glucose alone will produce the lesion (however the effect of hyperglycemia cannot be reduced to a simple formula (see below). The emphasis of Mirsky<sup>11</sup> and others on the rarity of this lesion in human diabetes would seem to have little relevance since obviously experimental conditions do not obtain and the stimulus whatever it may be probably acts over a period of years rather than days or weeks. Furthermore it is rare that a fresh newly discovered case of diabetes (i.e. the type in which one would expect hydropic change) comes to autopsy. Also if it be true as the work of Duff and Loreson<sup>9</sup> suggests that insulin can reverse this change even in the presence of hyperglycemia then one would expect the lesion to be rare in treated patients.

Speculation concerning possible causes for the selective destruction of  $\beta$  cells in the islets (see Chapter 3 for evidence that  $\beta$  cells are reduced in number in many cases of diabetes) was greatly stimulated by the finding that alloxan in proper doses causes such destruction (see Chapter 2a). The possible role of alloxan or a closely related substance in the etiology of diabetes has been elaborated particularly by Lazarow<sup>12-14</sup>. This author was responsible for demonstrating that a large dose of glutathione administered just before a diabetogenic dose of alloxan would protect the experimental animal from developing diabetes. As a result of this and similar observations he suggests that the specialization of the  $\beta$  cells for insulin synthesis may result in a localized depletion of glutathione in these cells. He points out that insulin is 12 per cent cystine and depends on the S-S group for its activity and that the cystine can be formed by oxidation of cysteine which is also a constituent of glutathione. Because of this situation there may be competition for the cysteine reaching the  $\beta$  cells since it is used for synthesis of both insulin and glutathione. As a consequence of this competition Lazarow suggests there may be less cysteine available in the  $\beta$  cells for glutathione synthesis than is the case with other cells. It has been further calculated that if the cysteine contained in glutathione could be made available for insulin synthesis then the synthesis of physiological amounts of insulin could deplete the  $\beta$  cells of glutathione and thereby render the enzymes of these cells more susceptible to alloxan. Lazarow

of particular reference to glutathione the SH-containing enzymes and the effects of alloxan

higher insulin requirement than pancreatectomized animals were able to live longer without insulin and did not develop ketosis and coma. As a result of their observations these authors put forth the hypothesis that there is secreted by the pancreas a second endocrine factor which acts to increase the blood sugar but to prevent ketosis in the insulin-deficient animal. More recently the H G factor has been studied by Sutherland and co workers<sup>35, 37, 38</sup> and by Pincus.<sup>39, 40</sup> Sutherland<sup>41</sup> has recently reviewed the subject concluding that the factor is a protein and in all probability a hormone which raises the blood sugar by promoting glycogenolysis in the liver. Sutherland and de Duve<sup>37</sup> were able to obtain the substance not only from commercial insulin preparations but also from the pancreatic tissue of several species and from the upper three-fourths of the gastric mucosa of the dog. Since they found a normal or increased amount in the sclerosed pancreas after ligation of ducts (which destroys the acinar tissue but allows the islets to survive) and normal amounts in the pancreas of alloxan-diabetic animals they suggested that it was entirely possible that the substance was produced by the  $\alpha$  cells. They noted however that the presence of an apparently identical factor in the upper part of the gastric mucosa of the dog raised the question of specificity unless it could be shown that the

by this method<sup>42</sup> hence there must be some histochemical difference between the two cell types.

Gaede *et al*<sup>43</sup> have described a single dog to which some weeks after ligating the pancreatic ducts they gave alloxan thus producing islets consisting almost solely of  $\alpha$  cells embedded essentially in fibrous tissue. From this sclerotic pancreas they obtained an extract which raised the blood sugar of a normal rabbit from about 90 to 119 mg per cent. They explained the feeble rise in blood sugar by the small size of the islets and concluded that the  $\alpha$  cells were indeed the sources of the hyperglycemic factor. Such an isolated observation though interesting is of course not conclusive especially since no control observations on extracts of other tissues are offered. It is perhaps conceivable that some nonspecific substance in the extract having an epinephrine-like effect accounted for the slight rise in blood sugar observed. However this seems to be the nearest approach to a pure extract of  $\alpha$  cells and seems to fit in with the work of others. Lerner<sup>44</sup> has set forth various hypotheses in relation to the possible importance of the H G factor and the total mass of  $\alpha$  cells in normal carbohydrate metabolism and in relation to insulin resistance. These speculations are stimulating and thought provoking but are as yet unsupported by sufficient evidence to make them acceptable.

Pincus<sup>45</sup> apparently with a similar hypothesis in mind gave insulin preparations with the insulin inactivated by heat and alkali to diabetic and normal persons and noted the most marked rise in blood sugar among

albumin and globulin fractions of the serum protein. At all ranges of serum protein concentration the diabetics showed a lower cystine protein ratio than the normals. These authors also found using sodium sulfate fractionation that 46 per cent of 40 fasting diabetics showed an albumin globulin ratio under 1.5 while some of 19 normals had ratios as low. Their diabetic patients showed further a striking variability in serum cystine and serum protein concentrations and in the cystine-protein ratio in contrast to a remarkable constancy in the normal individuals studied. The authors suggest that this variability may be related to the activity of insulin and to the state of control of the diabetes at the time the blood sample was withdrawn. Experimental confirmation of these data was lent by the finding of a low total cystine and low cystine protein ratio in animals with diabetes produced either by alloxan or pancreatectomy and by the tendency of injected insulin to raise the total cystine and the cystine-protein ratios toward their original levels.

To date of course all assumptions concerning the intracellular metabolism of the  $\beta$  cell are highly speculative. It is possible too that all such hypotheses may have to be modified in the light of Toreson's demonstration that in many if not all cases of so-called hydropic degeneration of the islands are due to actual intracellular deposition of glycogen. It is conceivable as indicated elsewhere that the presence of this glycogen is simply a nonspecific manifestation of storage as occurs in many other cells of the body. According to this point of view it would perhaps be no more surprising to find glycogen in the islet cells than to find it in the cells of the eye (see Chapter 13). Yet experimental evidence suggests strongly that in the  $\beta$  cells such glycogen deposits are the earliest manifestations of injury.

Toreson<sup>16</sup> makes the interesting suggestion that the glycogen in the islands and in the ducts may be an indication of regenerative activity. As noted elsewhere Duff and Toreson<sup>17</sup> have made the important observation that insulin in small doses may reverse this change even in the presence of hyperglycemia. They conclude that hypoinsulinemia is a much more important factor in producing the change than is hyperglycemia.

In view of the newer work on the hyperglycemic-glycogenolytic (H.G.) factor of the pancreas and the suggestion that this hypothetical hormone may be a product of the  $\alpha$  cells it now seems necessary to go beyond the  $\beta$  cells in considering the role played by the pancreas in diabetes. In 1923 Murlin and co-workers<sup>18</sup> noted the presence in extracts of pancreas of a substance which would raise the blood sugar and which they named glucagon.<sup>19</sup> Such a substance was encountered by various investigators as a contaminant of various American and Canadian insulin preparations.

H. G.

further substantiation of this evidence occasional mitotic figures can be found in the cells of the younger pigment free islands.

The pigment is not restricted to the island cells but affects the acinar tissues as well. The same evidence of regeneration is offered by the acinar cells as by those of the islands.

The well-established evidence of destruction and regeneration of parenchymal cells in the liver offers a striking parallel to the changes in the pancreas in hemochromatosis. Just as in the liver the parenchymal cells show every stage from newly formed pigment free cells through those containing hemofuscin and those containing hemosiderin to necrotic cells, the same steps can be traced in the acinar and island cells of the pancreas.<sup>44</sup>

The pancreas in diabetes is not simply the scorched field of an old battleground but is the actual field of conflict. It does not submit without a struggle to injury but endeavors to regenerate. This leads to the hope that in young people with their more resilient tissues proper treatment may remove the cause of injury and favor more or less complete restoration of the damaged islands.

Arteriosclerosis as an etiologic factor in diabetes is not given much weight in most discussions of the subject. However Moschcowitz<sup>45</sup> holds that hyperplastic and hyaline arterial lesions are more frequent in older diabetics particularly hypertensive ones than in nondiabetics of comparable age and that lesions of the islands (fibrosis and hyalinization) should be regarded as a capillary sclerosis extending from the arteriolar lesions.

## EXTRAPANCREATIC FACTORS

As noted at the outset an insulin deficiency may be due to (a) actual decrease in output of the pancreas due to a reduction in the quantity of islet tissue or (b) excessive demand for or destruction of insulin so that even though the pancreas is producing a normal or even supernormal amount of insulin the supply is inadequate to meet the demand. The latter condition seems to occur in the early stages of some forms of experimental diabetes although as previously emphasized pancreatic lesions can be demonstrated later and must be regarded as responsible for the permanence of the diabetic state. It should be remembered therefore that many of the so-called "extrapancreatic" factors listed below exert their effects sooner or later directly or indirectly upon the pancreas. In fact pancreatic damage is probably essential if a permanent diabetes is to follow.

**The Pituitary** — A possible role of the pituitary in human diabetes has been considered since the classical work of Housley in showing the ameliorating effect of hypophysectomy on experimental diabetes. The experimental production of diabetes in animals or man, whether by means of crude extracts<sup>46</sup> purified adrenocorticotrophic hormone<sup>47,48</sup> or purified growth hormone<sup>49,50</sup> seems to lead to early onset of (idiopathic or pharyngeal diabetes of Young) in which the  $\beta$  cells can



patients with labile diabetes. McQuarrie *et al*<sup>44</sup> report an interesting condition of familial hypoglycemia in which  $\alpha$  cells are said to be completely or almost completely absent from the pancreas.

If such an  $\alpha$  cell hormone exists it may of course help to explain many previously puzzling observations such as the smaller insulin requirements of dogs with total pancreatectomy as compared with those having a partial pancreatectomy<sup>45</sup> and the similarly reduced insulin requirements of totally pancreatectomized man as compared with natural diabetes.<sup>16, 17</sup>

However the validity of many of the experiments on which such conclusions are based has been questioned recently by Mirsky *et al*<sup>46</sup>. They hold that pancreatectomy in the alloxan-diabetic dog actually produces an aggravation of the diabetic state since the hyperglycemia, glycosuria and ketonemia observed during a period of fasting and exogenous insulin deprivation are greater after than before pancreatectomy in such an animal.

In any case the pathology of the pancreas in diabetes cannot be considered as static. As seen at any one time it represents a balance struck between the factors tending to produce degenerative changes of the islands and the regenerative powers of the pancreas. That these latter are by no means inconsiderable has been shown by the experimental work of Bensley<sup>47</sup> and his pupils and the observations of Boyd<sup>48</sup> and is evidenced also by the not infrequent occurrence of mitotic figures in islands when the epithelium has been injured.

Considerable regenerative activity may be seen in various types of experimental diabetes (see Chapter 2c). Rust<sup>49</sup> has described the remarkable fluctuations which can be produced in the total volume of the islands in the rat by means of various stimuli (see Chapter 3). Apparently repeated small doses of insulin may lead to a disuse atrophy of the islets.<sup>49</sup> It is conceivable of course that the fundamental defect in the diabetic is an inability to regenerate  $\beta$  cells at an adequate rate. Species differences in animals are well recognized for instance the immunity of the rat to pituitary diabetes is supposedly due to its ability to regenerate  $\beta$  cells.<sup>4, 10, 5</sup>

New formation of islands from intralobular duct epithelium has been described by Burkhardt<sup>50</sup> in 2 cases of carcinoma of the ampulla involving the head of the pancreas giving a picture similar to that of duct ligation. Most islands were atypical and showed degenerative changes. Both cases showed clinical diabetes. In both cases the carcinoma probably antedated the diabetes.

The important role played by hemochromatosis in elucidating the process one of us has already stressed. We find variation in involvement of the islands (in hemochromatosis) ranging from the remains of islands represented by clusters of pigment loaded endothelial leuko-

formed to take the place of those destroyed by the pan-

Chapter 16) Here it becomes obvious that pituitary diabetes and adrenal diabetes must overlap since it is uncertain how much of the effect of crude pituitary extracts used in the past may have been attributable to the adrenotropic and how much to the growth factor. Presumably the diabetes produced by ACTH and the associated disturbance of uric acid metabolism is due to overproduction of steroid hormones by the adrenal cortex although this is not established.<sup>25, 26</sup> The recent observation of hydropic change (glycogen) in rabbits injected with cortisone by Kobernick and Mori<sup>26</sup> is an important lead (cf. Chapters 16 and 25 and Figs. 13 and 14).

Because of the associated upset in purine metabolism produced by ACTH and manifested by increase in urinary excretion of uric acid and because of the alleviation of this type of experimental diabetes by glutathione Conn<sup>27</sup> has hypothesized that a purine metabolite exerting an alloxan-like effect is responsible for reducing the intracellular availability of free sulfhydryl groups which are necessary for the normal activity of many enzyme systems; that the combination of decreased intracellular concentrations of sulfhydryl groups and increased intracellular concentrations of purine metabolites impairs functional activity of the insulin producing cells of the pancreas (the product of which is rich in cystine) and also interferes with peripheral glucose utilization by inhibition of those enzyme sys-

tem.

tered in the adrenals in human diabetes whether functional or anatomical may be entirely nonspecific and secondary to a state of stress in the sense of Selye.<sup>47</sup> In any given case therefore it may be impossible to say whether the adrenals have any primary or etiologic significance. The effect of removal of tumors however is convincing.

Recent evidence suggesting that adrenal cortical function may actually be diminished in diabetes is given in Chapter 16, page 202.

**The Thyroid.** Hounsley<sup>48</sup> has shown that a temporary or permanent diabetes can be produced by administration of thyroid extract in animals with already damaged pancreases (Chapter 25). Clinically it is recognized that hyperthyroidism developing in a diabetic may cause exacerbation of the diabetes and myxedema may lead to amelioration. Balfour and Sprague<sup>49</sup> give illustrative examples. Ricketts<sup>50</sup> suggests that hyperthyroidism appearing in a patient predisposed by heredity or other factors to diabetes may precipitate the disease in persistent form. Increased activity of the thyroid would appear to be an excellent example of an extra-pancreatic factor producing an excessive demand for insulin since presumably the effects of the thyroid are exerted solely through its influence in speeding up the rate of metabolism.

**Neurogenic Diabetes.**—The occurrence of emotional glycosuria and the sugar puncture of Claude Bernard point to a close relationship between

usually be found followed by a later permanent metahypophyseal phase in which the  $\beta$  cells are destroyed.<sup>46</sup> Whether the primary injury to the  $\beta$  cells is due to functional overstrain because of an increased demand for insulin to injury by an alloxan like substance appearing as a result of a simultaneous aberration of uric acid metabolism to glycogen deposition or to other unknown factors is not clear at the present time. Anderson and Long<sup>47</sup> have shown that growth hormone apparently inhibits the production of insulin by the pancreas. It is also possible that the anterior pituitary produces a specific inhibitor of hexokinase thus causing fundamental interference with carbohydrate metabolism.<sup>48-50</sup> The hypothesis oferner<sup>42</sup> that the growth hormone may be alphetotropic is mentioned above (p. 295). Clinical evidence that the pituitary may play a rôle in some cases of human diabetes is provided by the observations of White<sup>6</sup> on increased stature among diabetic children (although Rosenbusch<sup>8</sup> could not confirm this finding in Switzerland) and by the well known increased incidence of diabetes in acromegaly.

Bennett and Evans<sup>44</sup> sum up the experimental evidence for involvement of the pituitary in diabetes as follows. The four observations which offer the strongest support for the concept that a balance of hormones is involved [i.e. in carbohydrate metabolism] and that the balance is upset in the metabolic disturbance of diabetes mellitus are first the marked hypersensitivity to insulin that develops in hypophysectomized animals and that cannot be accounted for by secondary hypofunction of the other

ph ysectomy of deprecantize l animals and the ability of such dou is up r ated animals to survive for long periods of time without insulin and fourth the production of temporary or permanent diabetes mellitus in normal animals with crude hypophyseal extracts and reinjection of severe diabetes mellitus in hypophysectomized pancreatectomized animal in such extracts

3) et) or discussion of the pituitary may be found in Chapters 16 and 2)

createctomized rats.<sup>45</sup> Also an ameliorating effect on human diabetes has been observed with the onset of Addison's disease.<sup>46</sup> Additional evidence on the well known association of diabetes with tumors of both

diabetes has been produced in rats with human insulin by Forsham *et al* and by Conn using ACTH (References in

diabetics than in the general population.<sup>42</sup> Taub *et al*<sup>43</sup> have claimed to differentiate two groups of diabetics on the basis of liver function tests: the juvenile or insulin deficiency type and the adult or hepatic dysfunction type which would presumably correspond to Himsworth's<sup>4</sup> insulin-sensitive and insulin-insensitive types (see below). It is not by any means clear that the liver is the major factor in the second group.

It is unlikely that the liver *per se* is of great importance as an etiological factor in the disease. As Himsworth<sup>4</sup> points out it is usually necessary to destroy practically the entire liver before significant changes in carbohydrate metabolism are produced. Likewise it is not clear that the lipotropic factors (lecithin, choline, lipocystine) are concerned in the etiology of diabetes.<sup>1</sup>

**Trauma and Stress** Direct trauma in the sense of injury to the pancreas sufficient to destroy the greater part of the organ is rarely a cause of diabetes.

One of the few evidences for traumatic origin of diabetes is offered by Wells' case<sup>44</sup> that of a tenister, aged thirty-two years, who was run over by his wagon. Four and a half months after injury he was brought to the hospital stuporous, seriously ill and emaciated. It was learned that since the accident he had developed polydipsia and pruritus. Neither blood nor urine was tested for sugar as he was moribund. At autopsy the pancreas weighed 122 grams, was irregular and felt as though filled with small stones. The duct was not completely obstructed. No concretions were present outside the pancreas.

On microscopic examination fibrosis was extreme, with only traces of acini and a few shrunken, partly fibrosed islands. Throughout masses of calcified material were surrounded by a dense fibrosis. Most of the calcium appeared to be deposited as a result of necrosis of parenchyma. Over 70 per cent of the pancreatic tissue had been replaced in this way. Hence there was inadequate anatomical basis for the diabetes.

Although direct trauma to the pancreas is hardly ever a convincing cause of diabetes, there has been a tendency recently to broaden the concept of trauma to include injury to other parts of the body, severe illnesses, and even psychic stress. Selver<sup>45</sup> has suggested that diabetes may be one of the diseases of adaptation, and this concept has been enlarged upon by others.<sup>46</sup> It has certainly been amply demonstrated that emotional glycosuria occurs<sup>47</sup> and it may well be that this phenomenon is part of an alarm reaction or adaptation syndrome mediated perhaps through either the adrenal medulla<sup>48</sup> or cortex<sup>49</sup> or both. McArthur *et al*<sup>50</sup> have shown that the rate of urinary excretion of corticosteroids during diabetic acidosis may be 2 to 5 times as rapid as after recovery.

However, as noted above, diabetes seems to decrease during war time in spite of the associated stress.

**Obesity and Diet** The importance of obesity, usually an evidence of chronic overeating, as a predisposing factor in the production of diabetes

the central nervous system and the pancreas. Intermediate action of the adrenals and pituitary in the case of emotional glycosuria is quite possible, i.e., a stress phenomenon in the sense of Cannon<sup>70</sup> and Selye.<sup>67</sup> The World Wars, in spite of tremendous emotional strain on millions of persons, did not provide any noticeable increase in the number of diabetic cases; in fact, a decrease has been reported, presumably due chiefly to the prevalent undernutrition.

Lesions in the vicinity of the hypothalamus have been reported in a number of cases of diabetes.

An ependymal cyst of the third ventricle was found in a diabetic by Byrom and Russell.<sup>71</sup> Worthv of note is the observation of Gissel<sup>72</sup> that severe head injuries almost always produce a transient hyperglycemia. Davidson and Allin<sup>73</sup> found higher blood sugar values after injection of glucose in 12 patients with concussion and in 18 with skull fracture. However, these observations are not confirmed by other neurosurgeons (cf. Joslin<sup>74</sup>). At any rate, glycosuria following trauma may well be a non-specific stress phenomenon (*see below*).

Further evidence of the association of changes in the central nervous system and glycosuria has been brought out by Vonderahe.<sup>75</sup> Three cases presented hemorrhage affecting the tuber cinereum or hypothalamus with subsequent glycosuria; one case of diabetes of some duration showed a cyst of the pulvinar of the thalamus, and one case of capillary hemorrhage in the hypothalamus was accompanied by terminal hyperglycemia. One case of diabetes recovered following removal of a meningioma impinging on the fourth ventricle.<sup>76</sup>

Blackford and Venable<sup>77</sup> report 2 cases of paresis with diabetes in which they believe the diabetes was secondary to the syphilitic process.

As far as we are aware, the claims of Vonderahe<sup>75</sup> and of Morgan *et al.*<sup>78</sup> that a reduction in ganglion cells can be demonstrated in the paraventricular nuclei in diabetes have not been confirmed.

Whether hypothalamic lesions may act by way of the anterior pituitary or otherwise is not known. In recent years there has been a tendency to

not to emphasize the point that the release of sugar from the liver is responsible for the glucose tolerance curve, both normal and diabetic. Forham and Thorn<sup>79</sup> have suggested that determination of serum inorganic phosphorus simultaneously with a glucose tolerance test may give a clue to liver involvement in diabetes. Levy *et al.*<sup>80</sup> and Zimmerman *et al.*<sup>81</sup> have found evidence of *real* impairment of function and have also found fatty change and cirrhosis in a few biopsies (*see Chapter 6*). But Brown<sup>82</sup>, applying 8 standard liver function tests in 20 diabetics, found no evidence of impairment (choice of tests and interpretation of them are obvious bases for disagreement). Also, there seems to be no evidence that Laennec's cirrhosis of the liver is more common in

whose disease had been discovered during the normal period of growth (growth-onset type) while relatively high values were found in subjects who had reached full stature prior to discovery of the diabetes (maturity-onset type). The authors speculate as to the relative importance of the growth hormone in the two types.

The effect of prior diet has been studied extensively by Hunsworth<sup>4</sup> who believes that the incidence of diabetes can be related to the amount of fat in the diet although the mechanism by which a high fat diet might bring on diabetes is obscure just as the predisposing effect of obesity *per se* is obscure. The idea that a high carbohydrate diet may have etiological significance seems less popular than in previous years in spite of the demonstration that massive amounts of glucose will produce diabetes in animals. (See next section below.)

In this connection the work of Houssay and Martinez<sup>20</sup> is of interest. They showed that a diet high in certain types of fat (lard and ox fat) would greatly increase the mortality of rats from alloxan diabetes. The effect of diet on the actual volume of islet tissue is noted elsewhere (p. 22). The occurrence of diabetes in experimental obesity associated with hypothalamic lesions is mentioned in Chapter 25 (p. 286).

**Hyperglycemia.**—An excellent discussion of the possible pathogenetic role played by hyperglycemia in both experimental and human diabetes is given by Lukens.<sup>45</sup> He concludes that it is impossible to state flatly that hyperglycemia is or is not harmful since any injurious effect may depend on co-existent circumstances. For instance under certain experimental conditions a diabetes in the early reversible phase may be cured by either insulin or phlorhizin both of which seem to lower the blood sugar by different mechanisms. Also diabetes may be produced by excessive administration of glucose to cats. On the other hand if hyperglycemia *per se* is harmful it is not clear why the metahypophyseal diabetes of dogs does not show a rapidly increasing severity nor why mild diabetes in man does not usually become more severe nor why the diabetes associated with pheochromocytoma (Chapter 16) is so readily reversible. The work of Duff and Toreson<sup>46</sup> indicating that insulin treatment may reverse hydropic change (glycogen infiltration) of the islets even in the presence of continuing hyperglycemia suggests that some other factor *e.g.* hypoinsulinemia may be more important. On the whole it would seem that an agnostic rather than a dogmatic point of view on this subject is desirable.

**Infection.** While the temporary unfavorable effect of infectious diseases in diabetes is established beyond all equivocation as a direct cause of the disease it is of little importance. Histological resemblances between pancreas and parotid have led to special interest in mumps,<sup>47,48</sup> but this disease can be but rarely blamed for diabetes.

Severe toxic injury to the islands in the course of various infections may produce either transient glycosuria or more rarely true diabetes. Root and Warren<sup>49</sup> have called attention to the actual necrosis of insular epithelium

has been well emphasized by Joslin<sup>24</sup> and others. Obesity may be regarded as indubitable evidence of abnormal food intake and hence of abnormal strain on the islands of Langerhans.

Table 47 illustrates the frequency of obesity.

TABLE 47 VARIATION FROM NORMAL OF MAXIMUM WEIGHTS AT OR PRIOR TO ONSET OF 1000 CALORIES OF TRUE DIABETES CALCULATED FOR HEIGHT AGE AND SEX (JOSLIN<sup>24</sup>)

Age years	Number of cases	Percentage in normal average zone (+5 to -5 per cent)	Percentage of each decade	
			Below standard weight	Above standard weight
0 to 10	43	37	44	11
11 to 20	84	39	29	32
21 to 30	112	19	10	71
31 to 40	172	6	5	80
41 to 50	244	12	3	87
51 to 60	252	12	1	87
61 to 70	9	10	0	84
71 to 80	14	11		79

A striking fact is the usual reversibility of diabetes in the middle-aged obese subject i.e. the glycosuria may be expected to disappear if weight loss is adequate. In fact consideration of certain data leads to the startling conclusion that almost half of the diabetics extant could be removed from this category simply by cure of their obesity.<sup>26</sup>

The frequent association of diabetes with obesity especially in the middle-aged is a well known fact. In the classification of this group into insulin sensitive and insulin insensitive types the former or the so-called mild or unstable severe diabetes respond readily to insulin the latter usually middle-aged and obese and tolerating large doses of insulin without symptoms. The former he considers as possibly due to insulin lack the latter to insensitivity to insulin. There is an obvious parallel between the latter group and animals in the adrenalectomized stage of pituitary diabetes. Of particular interest in this connection are the recent observations of Bernstein and Lawrence<sup>27</sup> who carried out insulin assay by a new method on the blood of two groups of patients one the young severe type with ketosis and requiring insulin to live the other the middle aged obese type without ketoacidosis and contraindications to insulin therapy. They found that while all of the former group were responsive to insulin the latter group were not.

Also important is the recent work of Wiens<sup>28</sup> who studied the extractable insulin as measured by a mouse convulsion or a mouse blood sugar method in a series of pancreases from diabetic and nondiabetic individuals. The lowest values relative to controls were found in diabetics.

Harris<sup>99</sup> holds the problem to be more complex than hitherto assumed.

7 per cent of the siblings of diabetics whose age at onset of the disease is less than 30 may be expected to develop diabetes by age 40 while only about 1.3 per cent of the siblings of those who develop the disease after the age of 30 may be expected to have diabetes by age 40. He suggests that many of the late onset type may be heterozygous for a gene which in homozygous form may give rise to the severe early onset type of case.

TABLE 48 INCIDENCE OF DIABETES IN THE FAMILIES OF DIABETICS PERCENTAGE OF CASES REPORTING HEREDITARY AND OR FAMILIAL TYPES OF FAMILY HISTORY RECENT CLINICAL EXPERIMENTS (1914-24)

	No of cases	Per cent with family history of diabetes
Joulin total children 1946	2191	35.0
Joulin 1946 Children of 20 or more years duration	249	55.0
Joulin 1941 Hospital cases	1619	41.0
Joulin 1920-1928	4831	25.6
Harach Pittburgh 1933	1000	44.5
John Chivellan 1938-1939	630	35.7*
Lawrence London to January 1933	5462	32.1
U. S. Veterans Bureau to August 1933	704	7.0†

\* Includes 7 cases with combined hereditary and familial history.

† Includes 35 cases with combined hereditary and familial history.

Barker *et al*<sup>100</sup> describe a family in which 3 boys all developed diabetes at an early age while 3 girls were spared. They suggest inheritance of the disease as a sex linked recessive with heterozygous parents.

## CASES WITH NO SIGNIFICANT PATHOLOGY

There remains to be explained the group of cases without definite anatomical change in the pancreas or other organs. Even the granules in the island cells may appear normal. In our series of 811 diabetic autopsies the pancreas was normal as far as could be determined in 271 or almost exactly a third of all cases. (In most of these the newer staining methods were not used.) No lesions in other organs were sufficient to explain the existence of diabetes although the pituitary was examined in only a small proportion. However no evidence of clinical disturbance other than the diabetic process was encountered.

These cases in which changes in the pancreas are absent or too trifling to merit consideration have always puzzled and irritated the pathologist. It



that may occur in such cases and also to evidences of regeneration. Of

prob-  
 is present in all cells of the body and catalyzes a key reaction by which glucose is phosphorylated. The anterior pituitary is thought to produce a specific inhibitor of hexokinase<sup>45,46</sup> also one of the actions of the oxygenated steroids of the adrenal cortex appears to be the enhancement and prolongation of the action of this pituitary hexokinase inhibitor.<sup>47</sup> It has been further held that a decrease in hexokinase activity could be demonstrated in the muscle and liver of the alloxanized rat.<sup>48</sup>

The possible rôle played by these reactions in the pathogenesis of human diabetes must await further work especially since others<sup>49</sup> have had difficulty in completely confirming the original observations of Cori *et al*.<sup>45</sup>

**Insulinase**—Recently Mirsky and co-workers<sup>50</sup> in a series of papers have described an enzyme system (insulinase) which inactivates insulin. Apparently fasting diminishes the liver insulinase and feeding reconstitutes it. The possible place of this enzyme in the physiology of human diabetes is not yet clear.

**Phosphatases**—Since these enzymes are of undoubted importance in carbohydrate metabolism especially in the dephosphorylation of glucose it is natural to consider whether they are involved in the pathogenesis of diabetes. Drabkin<sup>51</sup> considers that they are and holds that a relationship can be demonstrated between the level of the blood sugar and the phosphatase activities of the kidney, blood serum and liver. Also the apparent increase in liver phosphatases in alloxan diabetes and their restoration to normal suggest that these enzymes are of significance in the diabetic state. Bunting<sup>52</sup> found less than normal amounts of alkaline phosphatase in the kidney of alloxan-diabetic rats. Deane<sup>53</sup> has found alkaline phosphatase in the islets of Langerhans and has shown it to be present in greater concentration at the periphery of the islet in the rat.

**Heredity**—This is the most important predisposing factor of all. There is a statistically significant higher incidence of diabetes among blood relatives of diabetic patients than in the general population. The evidence and implications are discussed by White and Pincus.<sup>54</sup> They believe that the inheritance follows a Mendelian recessive pattern. When both parents have diabetes all the children may be expected to develop the disease if they live long enough when one parent has diabetes and the other is a diabetic carrier 40 per cent of their children may develop the disease when is one diabetic or a carrier mingles in individual who neither has diabetes nor is a diabetic carrier none of the children will have diabetes. The implications of the last statement from the public health point of view are tremendous. Outbreeding of the disease by the consistent union of descendants of diabetic patients with those of nondiabetic families is theoretically possible.

betes, in man and in the experimental animal. However, all forms of permanent diabetes in experimental animals are characterized by lesions of the islets of Langerhans, and it seems probable that irreversible human diabetes is also fundamentally pancreatic, i.e., essentially an insulin deficiency disease. No exact synonym exists to make a matter of fact.

er<sup>1</sup> has recently made an able defense of the unitary pancreatic view of human diabetes. The rôle of the H-G factor if any, in human and experimental diabetes is yet to be determined.

## REFERENCES

- [illegible]

is difficult to conceive of long-continued and profound functional pathology without organic change the field of mental disease excepted

One may guess of course that these cases fall into the group in which even though insulin secretion is normal the demand greatly exceeds the supply or in which the normally formed insulin may be inactivated (by insulinase?) It is more likely, perhaps that newer histological methods particularly silver impregnation the phosphotungstic acid hematoxylin method of Hultquist and Tegner, and Gomori's new methods (see Appendix) will show that many of these cases with apparently normal islets actually have a marked reduction in the number of  $\beta$  cells or an increase of  $\alpha$  cells or both (see Chapter 3) Also the total volume of the islet organ <sup>49, 50</sup> may be altered

It is possible that some other mechanism may be operative for instance a circulatory one Since the amount of insulin demanded fluctuates between wide limits some rapidly functioning regulatory mechanism must exist for the output of insulin Obviously the amount utilized in absorbing a heavy meal would produce marked hypoglycemia if secreted in a fasting period the intravenous glucose solution so frequently used by surgeons puts a heavy and sudden demand on the insulin producing mechanism yet this is readily met In the absence of prompt control we would swing ceaselessly and dizzily from hyperglycemia to hypoglycemia and back

This regulation may be accomplished by alterations in rate of secretion which is partially dependent on the amount of blood passing through the islands or by alterations in amount of blood flow which determines not only the nutrition of the island cells but the amount of insulin transported from the islands

In examining the living pancreas of the mouse by a modification of Covell's<sup>52</sup> method using transmitted light one of us (S W) found in the thinner portions of the pancreas reddish white spots which faded slightly from time to time and others appeared Subsequent histological examination indicated these spots to be islands of Langerhans Thus we may have an intermittent circulation of blood through the islands and a very simple and rapid means of regulating the output of insulin Such an intermittent circulation might be expected in view of the known intermittent circulation in the glomeruli of some animals the peripheral capillaries and elsewhere

Berg<sup>53</sup> using reflected light has reported similar results emphasizing the variation in the capillary pattern and the rapidity with which these changes occur

It is theoretically possible therefore that a circulatory disturbance might be responsible for failure of release of insulin from histologically normal islets However no such mechanism has been demonstrated

### SUMMARY

It is obvious that innumerable factors may come into play in producing a temporary diabetic state or in accentuating an already established one





pains to avoid even minor trauma and to protect any injury received even to a blister, from infection. So long as his diabetes is under control and his circulation adequate he has little more to fear than a normal person. Infection always makes an existing diabetes worse and inadequately controlled diabetes an ideal setting for rapid extension of an infectious process especially of the pyogenic type. As has been pointed out organisms of low pathogenicity as *Staphylococcus albus* and *E. coli* may be highly dangerous to the diabetic.

Uncomplicated physical injuries as fractures and clean wounds heal as well in the diabetic patient as in others. However suddenly changed habits of exercise and diet may complicate the management of the diabetes.

Acidosis and coma need not be a factor if adequate dietary control and insulin are utilized as needed. However any dietary indiscretion or inadequacy of insulin is dangerous. Infection is of major importance in precipitating coma. Impending coma impairs judgment and responsibility.

Arteriosclerosis usually gives ample warning of its presence in the arteries of the legs. Cleanliness and maintenance of warmth are essential to prevent gangrene. When arteriosclerosis involves the coronaries however a fatal occlusion may be the first inkling of its presence. Avoidance of sudden fluctuations of blood sugar and undue exertion are useful protective measures.

Occupations requiring long and irregular hours without opportunity for control of the diet and use of insulin should be avoided by the diabetic patient.

## INSULIN REACTIONS

Insulin reactions usually are not fatal. A man aged seventy years whose left leg had been amputated at mid thigh for gangrene sat up in bed. Feeling faint he called to others in the ward. "I'm having an insulin reaction and feel dead." His post mortem examination revealed an embolus in his pulmonary artery and a slightly elevated blood sugar. Case 32 772 supposedly died of an insulin reaction but the autopsy showed a fresh coronary occlusion. Case 36 597 died suddenly twelve days after a fracture of the femur. An insulin reaction was suspected but the autopsy revealed the pulmonary artery occluded by an embolus. Such cases could be readily multiplied. No death should be ascribed to insulin without an autopsy and blood sugar determination.

There are positive changes in death due to excess of insulin as have been described in Chapter 22.

Aside from fatal insulin reactions hypoglycemic coma, complete or partial, is of much significance. The clinical picture may simulate drunkenness. Hyperinsulinism may be precipitated by delay in obtaining food, by sudden amelioration of an infectious process, by mistake or carelessness in dosage, or by intent. The hypoglycemic person may lose consciousness wholly or in part. It is difficult to estimate at what point he ceases to be

## Chapter 27

### MEDICO LEGAL ASPECTS OF DIABETES MELLITUS

THE medico legal aspects of diabetes may be considered under four headings: the etiology of the disease in relation to trauma and occupation; special hazards of the diabetic state; effects of insulin; particularly hyperinsulinism; the diagnosis of the disease from post mortem material.

While one's sympathy may well be aroused by the plight of a diabetic patient who may believe his disease resulted from trauma or some occupational hazard or was aggravated thereby, any such claim should be most carefully scrutinized not only for the sake of scientific accuracy but also for the sake of the hundreds of thousands of diabetics who will be hampered in obtaining gainful employment should the risk and cost of compensation of certain of them prove too high.

Rigid control of specimens of blood and urine used in establishing the presence or absence of diabetes must be maintained.

#### ETIOLOGY

Trauma may be claimed but is not a significant factor in light of our

betes following head injuries may be based. Certainly temporary disturbances in carbohydrate metabolism may follow injury of any kind but this is to be regarded as a nonspecific reaction to stress. As noted in the preceding chapter no increase in diabetes was found in either World War.

Psychogenic origin for diabetes has not been proved in spite of the evidence that emotional factors cause exacerbation of the disease and might theoretically act as the precipitating factor in a predisposed person.

Trauma may lead to persistent infection as osteomyelitis<sup>4</sup> which might predispose to the development of diabetes.

Occupation is not known to be a significant factor in the causation of diabetes.

Extensive discussion of the medico legal aspects of trauma may be found in Joslin's book.<sup>2</sup>

#### SPECIAL HAZARDS

The chief of these are infection, acidosis and coma, and arterio-sclerosis. The diabetic must take special precautions against infection, taking

pains to avoid even minor trauma and to protect any injury received even to a blister, from infection. So long as his diabetes is under control and his circulation adequate, he has little more to fear than a normal person. Infection always makes an existing diabetes worse and inadequately controlled diabetes is an ideal setting for rapid extension of an infectious process, especially of the pyogenic type. As has been pointed out, organisms of low pathogenicity, as *Staphylococcus albus* and *E. coli* may be highly dangerous to the diabetic.

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There are positive changes in death due to excess of insulin as have been described in Chapter 22.

Aside from fatal insulin reactions hypoglycemic coma complete or partial is of much significance. The clinical picture may simulate drunkenness. Hyperinsulinism may be precipitated by delay in obtaining food, by sudden amelioration of an infectious process, by mistake or carelessness in dosage or by intent. The hypoglycemic person may lose consciousness wholly or in part. It is difficult to estimate at what point he ceases to be



responsible. Careful clinical study, reinforced by blood sugar determinations will aid in establishing this.

Numerous examples of the medico-legal importance of insulin reactions are given in the discussion in Joslin's book.<sup>2</sup>

### THE DIAGNOSIS OF DIABETES MELLITUS FROM POST MORTEM MATERIAL

Post mortem diagnosis of diabetes while sometimes impossible may frequently be made definitely. It must be remembered the adequately treated diabetic may show no evidence of the disease. Post mortem urine sugar determinations may be helpful as may the presence or absence of acetone bodies. Absence of sugar in the urine should not be relied upon if the urine is heavily contaminated with bacteria or if marked acidosis exists as renal tubular damage may have caused a renal shut-down.

The post mortem blood sugar is of great value. In the absence of gross contamination the diminution of blood sugar may be very slow. Thus in Table 49 we see the results obtained from defibrinated heart's blood kept at room temperature for varying lengths of time before analysis. Although there was a sudden marked initial drop in one case the subsequent decrease was slow in both cases.

TABLE 49 DECREASE IN BLOOD SUGAR OF DEFIBRINATED BLOOD AT ROOM TEMPERATURE

Time in hours	Blood sugar in Mg per cent	
	Case No 5062	Case No 3729
<i>Ante mortem</i>		
3.5	400	
4.5		230
<i>Post mortem</i>		
1.25		300
2.0	46	
4.0		260
4.5	44	
5.5		220
6.5	44	
8.0	43	
10.0	40	
13.0	24	
19.0		140
23.5	16	
26.0		130
28.0	16	

In Table 50 a number of ante-mortem and post mortem blood sugars are compared. Thus the blood sugar may remain abnormally high and an evidence of diabetes for some hours after death. (The fact that some of the post mortem values are higher than the ante-mortem may perhaps be explained by some of the considerations mentioned below.)

TABLE 30.—COMPARISON OF ANTE-MORTEM AND POST-MORTEM BLOOD SUGAR VALUES

Pathological No.	Ante-mortem blood in gnr	No. of hours to death	No. of hours to autopsy	Post-mortem blood in gnr
15079	250 mg %	3	1 25	190 mg %
16771	1000	2 5	0 05	780
17720	920	Few	1	940
18159	210	Few	2 5	240
18153	940	4	2	210
19042	270	0 5	1	320
19342	990	3	0 1	1000
20173	130	5	1 5	10
21531	340	5 75	1 25	200
21682	320	4	1 5	220
22683	240	Few	1	80
23085	830	4	2	790
24049	340	Few	2 5	250
2515	100	Few	1 25	270
27095	190	1 5	3 5	320
28681	340	Few	2	320
29894	150	4	1 31	100
29761	690	Few	0 60	440
11954	290	Few	1	230
12990	520	12	5	290
14834	180	Few	4	160
11864	370	2	1	520

It is quite important that blood be drawn from the *left* side of the heart at autopsy. Blood in the right side may show high levels of reducing substances presumably due to proximity of the column of blood to the liver and diffusion of glucose from breakdown of glycogen in the latter organ. Hamilton Paterson and Johnson<sup>8</sup> state that values over 300 mg per 100 ml in the left heart may be taken to signify ante-mortem hyperglycemia. However, Hill<sup>6</sup> and Fisher<sup>7</sup> caution that agonal release of epinephrine from the adrenal glands may lead to a misleadingly high terminal blood sugar even in the left heart.

Naumann<sup>4</sup> has emphasized the value of using cerebrospinal fluid (obtained by cisternal puncture). Presumably the rate of glycolysis is less than that of blood. Probably also agonal changes are less marked. Here a level of over 200 mg per cent is probably significant. The presence of acetone suggests coma as the cause of death.

Histological study may reveal changes in the pancreas but as was pointed out in Chapter 4 these may not be pathognomonic of the disease. Cell counts by the newer methods (see Appendix) may be of value. It seems reasonable to state that if the ratio of  $\beta$  to  $\alpha$  cells can be shown to be less than 3 a presumptive diagnosis of diabetes can be made. Torsion<sup>9</sup> holds that the demonstration of glycogen in the islands is positive morphological evidence of diabetes. Degranulation of the  $\beta$  cells alone is apparently regarded by Bell<sup>10</sup> as a reliable diagnostic criterion. Conceivably a large terminal infusion of glucose might be misleading since Paterson<sup>8</sup> was able

to produce degeneration of  $\beta$  cells in experimental animals in fifteen minutes by massive infusion of glucose

In the inadequately treated diabetic, deficient liver glycogen together with glycogen in the liver cell nuclei and the renal epithelium may give the clue. Much glycogen may be present in the kidney in von Gierke's disease. That the carbohydrate content of liver tissue changes but little after the early post-mortem alteration is shown by the following table taken from Popper and Wozniak.<sup>11</sup>

TABLE 51.—TOTAL CARBOHYDRATE CONTENT OF LIVER IN RELATION TO TIME POST MORTEM<sup>12</sup>

Hours post mortem	Content	Twenty-four hours later at room temperature	Twenty-four hours later at icebox temperature
18	0.83	0.84	0.90
17	1.07	1.08	1.10
15	0.37	0.71	0.75

At this point it is well to emphasize that special alcoholic fixatives while useful are not necessary for the demonstration of glycogen<sup>13</sup> (see Appendix). Thus glycogen may be looked for in old paraffin blocks or in stored tissue.

Other lesions which are to say the least strongly suggestive of diabetes and which may be conclusive if added to other findings are intercapillary glomerulosclerosis in the kidneys (Chapter 12) and capillary microaneurysms in the retina (Chapter 13).

As an example of the conclusiveness with which a post mortem diagnosis of diabetes can be made under favorable circumstances the following case observed by one of us (P.L.) may be cited. The patient was a woman of fifty who died of a coronary occlusion. She was comatose during the last few hours of life having been taken moribund to a hospital. No suggestion of diabetes appeared in her history. At the time of autopsy the tissues had a sweetish odor (probably acetone). Urine taken from the bladder showed large amounts of sugar and acetone. Microscopic examination revealed the following: abundant glycogen in the renal tubules; occasional small granules of glycogen in the  $\beta$  cells of the islets of Langerhans; a  $\beta$  to a cell ratio of approximately 2.7 by the Gro-Schultze method; and typical intercapillary glomerulosclerosis in the kidneys. There were thus no less than five significant findings, any one of which might be considered as adequate presumptive evidence of diabetes while all five taken together establish the diagnosis beyond doubt, regardless of the negative clinical history. Incidentally of course a coronary occlusion in a woman of fifty is in itself sufficient to raise a very strong suspicion of diabetes (cf Chapter 9).

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## Chapter 28

# A UNIFYING CONCEPT OF THE PATHOLOGY OF DIABETES MELLITUS

At first sight the protein and heterogeneous manifestations of the histopathology of diabetes and its complications as described in the preceding chapters seem utterly unrelated and confusing. The desirability of attempting to devise a unifying hypothesis is obvious. Friedenwald has made an interesting start toward such a concept in relation to the capillary lesions of the eye and kidney. It is the purpose of this chapter to suggest that his hypothesis may be extended to embrace practically all the important lesions characteristic of diabetes.

Friedenwald<sup>1</sup> in one of his brilliant papers describing capillary microaneurysms of the retina emphasized the extraordinary frequency with which these lesions occur in association with intercapillary glomerulosclerosis in the same individual. He pointed out (as did Ashton<sup>2</sup>) that the staining reactions of the hyaline deposits in both diseases were similar and cited the suggestion of McManus<sup>3</sup> that the hyaline of the renal lesions may represent a polysaccharide deposited from the circulating blood. He also noted that Jacobs<sup>4</sup> had described in the blood of diabetics a mucopolysaccharide the concentration of which closely paralleled that of blood glucose in response to insulin and cited the work of Pirani *et al.*<sup>5</sup> on amyloidosis in scorbutic guinea pigs as indicating a possible relation of capillary fragility to a disturbance of polysaccharide metabolism. These bits of evidence although admittedly tenuous suggested to Friedenwald that a more extended analysis of mucopolysaccharide metabolism was justified in relation to diabetes and diabetic retinopathy.

Reflection suggests that most of the characteristic tissue changes seen in diabetes may be interpreted in terms of disturbances of metabolism (or structural organization) of a simple polysaccharide (glycogen) or of the more complex carbohydrates known as mucopolysaccharides. In this connection one may consider changes in the pancreas, the larger arteries, the kidney, the eye, and the skin.

The two most important lesions of the pancreas are hydropic change and hyalinization of the islets. If it be assumed that the work of Torson<sup>6</sup> cited in Chapter 3 is generally applicable then hydropic change must be regarded as due at least in part to the deposition of glycogen. If one places the amorphous substance in hyalinized islets in the category of amyloid (see Chapter 3) and if one accepts the view of Hass<sup>7</sup> of Jones

and Razier,<sup>8</sup> and others that amyloid may consist at least in part of mucopolysaccharide then one may perhaps consider hyalinization of the islets as due to the deposition of a polysaccharide-protein complex from the blood stream.

No adequate evaluation or even description of the vascular lesions of diabetes is possible without consideration of the properties of the little-understood ground substance and of basement membranes. Several excellent papers based on newer histochemical methods have appeared within the last year or so on this subject. Many of these are included in the New York Academy of Sciences Symposium on "The Ground Substance of the Mesenchyme and Hyaluronidase." Gersh and Catchpole<sup>10</sup> consider the ground substance of connective tissue and the homogeneous component of basement membrane to be closely related substances containing glycoproteins which form an optically homogeneous medium of fluid to gel-like consistency. These substances infiltrate and enclose a network of oriented fibrils. They are probably polymerized and presumably structurally organized on a submicroscopic level and according to these authors the degree of polymerization varies depending on age, activity, and degree of pathologic change. The chemistry and distribution of these substances is reviewed by Meyer,<sup>11, 12</sup> and some of the histochemical properties are discussed by Dempsey *et al.*<sup>13</sup> Durin Reynolds *et al.*<sup>14</sup> Lablond<sup>15</sup> and Ritter and Oleson.<sup>16</sup>

In various theories and discussions of arteriosclerosis of the larger arteries the ground substance has figured prominently. A swelling of the ground substance of the intima is an integral part of the intubation theory of Virchow and Aschoff (*see* Chapter 9). Recent papers which have emphasized its importance include those of Laber,<sup>17</sup> of Meyer,<sup>18</sup> and of Rinchuse and Greenberg.<sup>19</sup> It is entirely possible if not probable that the earliest lesion of atherosclerosis involves a subtle physicochemical change in the metachromatic gel or fluid which seems to encase the collagenous and elastic fibers of the arterial wall.

As regards the medial calcification of Monckeberg it may be said that the earliest human lesion is unknown although calcium salts often seem to be deposited first in elastic fibers. The medial calcification produced in animals by excessive doses of vitamin D has been studied in an effort to throw some light on the question and it is interesting that at least two authors<sup>20, 21</sup> describe the earliest visible change as a swelling of the interstitial ground substance.

Various points of view concerning intercapillary glomerulosclerosis or the Kimmelstiel-Wilson lesion of the kidneys have been discussed in Chapter 12. Regardless of whether one considers the amorphous hyaline material to be intercapillary or intracapillary, to be a deposit or a thickening of a pre-existing membrane, there can be no doubt that it is closely related to the substances under discussion. As noted in Chapter 12, McManus has shown that the characteristic staining qualities of this material are removed

by treatment with pectinase and he suggests that the deposit may be derived from a polysaccharide circulating in the blood. In this connection it would seem desirable to pursue the studies of Jacobs<sup>4</sup> and others<sup>5, 6</sup> on the circulating mucopolysaccharides of the blood plasma. Jacobs<sup>4</sup> studying the bound glucosamine of the blood in diabetic patients concluded that the mucopolysaccharide yielding this glucosamine was somehow involved in the diabetic defect and that it responded to insulin in roughly the same manner as glucose itself as far as the direction, speed and magnitude of the response were concerned.

The masterly studies of Friedenwald<sup>7</sup> on the eye have been cited in Chapter 13. As noted above it was he who suggested a close relationship between the capillary lesions in both the glomeruli and the retina. Certainly both lesions involve capillary basement membranes and there appears to be considerable similarity between the hyaline nodules in the glomeruli and the advanced microaneurysm of the retina. The presence of true neovascular dilatation of glomerular capillaries is not so clear however<sup>8</sup> although Allen<sup>27</sup> describes some degree of capillary dilatation as characteristic of the renal lesion.

The relation of basement membrane and capillary cement substance to capillary fragility is not clear. However that such a relation is present has been suspected since the classical work of Wolbach<sup>28</sup> on the intercellular substance in experimental scurvy.

Finally as regards the skin certain random observations in this laboratory (cited in Chapter 9, page 146) have suggested a possible increase in basophilic ground substance in the corium in some cases of diabetes. As noted there Linn<sup>11</sup> has described what appears to be the same substance under the name of collagen.

Recently Altshuler and Anguine<sup>29</sup> have published an interesting survey of the possible role of acid mucopolysaccharide in degenerative diseases of connective tissue with special reference to the chronic inflammation of Rosette<sup>30</sup> and Fpinger<sup>31</sup>. They point out the probable importance of acid mucopolysaccharides in diseases involving connective tissue and characterized by such substances or processes as hyaline amyloid, sclerosis and fibrinoid degeneration. Obviously many of the changes occurring in the diabetic organism would fall within this group of diseases.

It seems likely that the newer physicochemical methods for studying the finer structure of connective tissue reviewed recently by Gross<sup>3</sup> may throw light on this important problem. An extension of the use of the electron microscope from normal to diseased connective tissue has recently been made by Gale<sup>32</sup>.

In summary it appears that Friedenwald's concept may be extended to encompass all the major lesions characteristic of diabetes in the sense that all of them involve either simple polysaccharides (glycogen) or polysaccharide complexes (mucopolysaccharides) the latter probably often linked

to proteins. The hypothesis is admittedly tenuous but may prove useful if supported by future investigations.

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## APPENDIX A

### AUTOPSY AND STAINING METHODS

THROUGH careful studies made post mortem on the bodies of diabetic patients the clinical information obtained during the course of their disease can be correlated with the anatomical findings and the knowledge thus gained made available for other sufferers. As Joslin says: "An operation during life is attended by pain and is for the benefit of the individual; an operation after death is free from pain and is for the benefit of humanity."

Clinical observation and evidence at autopsy suggested the correlation of diabetes mellitus with diseased islands of Langerhans. Clinical observation, examination of surgically removed pancreatic tissue and autopsy proved that the islands produced insulin. The same methods may some day enable us to unravel the as yet but dimly understood etiology of the disease.

To quote from the motto on the wall of the post mortem room: *Mortui vivos docent* — the dead teach the living. The responsibility for the success of the autopsy rests directly upon the pathologist. His is the duty of seeing that all possible information is gleaned. His is the duty of seeing that the autopsy is performed in a seemly manner and that the needs of the undertaker are sympathetic ally considered. If he neglects the first it is futile for the internist to obtain permission for the autopsy. If he neglects the second it is impossible for the internist to obtain permission. An operation performed after the patient's death deserves as dignified surroundings as one before death.

There are certain features which must be especially considered in autopsies on diabetic patients. The weight of the patient is important and this is easily obtained. A small chain hoist is hung from the ceiling and a spring scale (Chatillon) suspended from this with a spreader and four hooks below. The truck on which the body lies is grasped by the hooks and the whole raised by the chain hoist. A tire hand previously set for the weight of spreader, hooks and truck permits direct reading of the net weight.

Since the pancreas is extremely labile and since glycogen in tissues rapidly hydrolyzes it is essential that the autopsy be performed as soon after death as practicable. If any long period of delay is anticipated the body should be placed in the cold chamber promptly.

The following autopsy technique is used in this laboratory. A useful primary incision is the Y type from over each acromial prominence to the sternum at the level of the third ribs and thence in the mid line to the pubis passing just to the left of the umbilicus. This affords an excellent

exposure and yet burial may be carried out in less than 1 hour, with no trace of the incision visible.

In an autopsy on a diabetic the pancreas should be removed first. A good exposure is obtained by cutting through the greater omentum. The pancreas is freed at the tail and stripped upward until the head, avoiding all unnecessary trauma or compression. The part of the pancreas most closely adherent to the duodenum is cut away. If the pancreas is found to have any abnormalities the gland has been noted and the entire pancreas portion of the pancreas is removed and weighed. Sections 1 to 2 mm in thickness are cut and placed in the various fixatives: skin, heart, striated muscle, and liver. The fixative is appropriate for subsequent staining for glycogen.

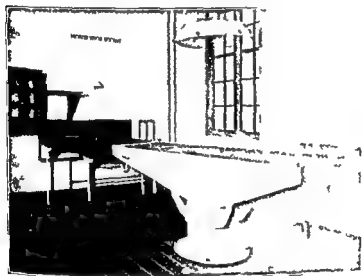


FIG. 112.—Post-mortem room for the Deaconess and Lutheran Memorial Hospitals, illustrating grid-top-type table with a retractable top.

The autopsy may then proceed in the normal manner. Largent has given excellent accounts of the various procedures. As soon as the heart is exposed a sample of blood is taken for blood sugar and nonprotein nitrogen determination. Cerebrospinal fluid may also be taken by lumbar or cisternal puncture.

Another variation from the usual routine is careful dissection of the remaining pancreatic tissue from the duodenum. This is weighed its weight added to the other portion and sections are taken.

A sample of bladder urine may be taken for glucose determination. A great assistance to the undertaker is the ligation separately of the in-

## AUTOPSY AND STAINING METHODS

nominate left common carotid and left subclavian arteries as well as the external iliacs. The ends of the ligatures are left outside when the primary incision is sewed up.

If permission has been given to open the head the carotid and vertebral arteries should also be ligated just within the cranium cavity.

Routine fixation is most satisfactorily accomplished in Zenker's fluid and in 10 per cent neutralized formalin. Absolute alcohol or better chilled Rossmann's fluid\* is used for all tissues in which the glycogen content is to be studied. Chilled acetone may be used if enzyme studies are contemplated. Helly's fixation is satisfactory for the hypophysis although hematoxylin and eosin after formalin give good results. Mann's stain as modified by Dorothy Russell gives good differentiation of anterior lobe cells but has not in our hands given as consistent results as Biggart's parrot blue-eosin<sup>2</sup> (method below). Lillie's<sup>3</sup> eryocyanine-saffranin or Crooke's<sup>4</sup> modification of Mallory's aniline blue stain.

The following additional fixing fluids may be used for the pancreas:

- 1 Bouin's fluid (especially useful for Gomori's stains)
- 2 Zenker's fluid without acetic acid
- 3 Helly's fluid
- 4 Regaud's fluid
- 5 Formol sublimate (formalin 1 part mercuric chloride 3 parts water 3 to 6 parts)
- 6 Carnoy's fluid
- 7 Champy's fluid

Potassium dichromate  
Chromic acid  
Osmic acid

## 8 Zenker osmic acid

3 per cent	21 cc
1 " "	21 "
2 " "	12 "

Zenker's fluid without acetic acid  
Osmic acid

## 9 Osmic chromic fluid

2 per cent	20 cc
	5 "

Osmic acid  
Chromic acid  
Acetic acid

2 per cent	4 cc
1 " "	30 "
glacial	1 "

All pancreatic tissue to be fixed in fluids containing osmic acid should be freed from fat as far as possible. Oil of cedar rather than chloroform is best for clearing the pancreas. Dioxane gives good results.

\* Rossmann's fluid to 9 parts of saturated picric acid in absolute alcohol add 1 part of concentrated formalin just before use. Stock solution is kept in the icebox and specimen bottle containing the tissue is immediately returned to the icebox. This chilling tends to prevent the pinching up of glycogen on one side of the cell due to rapid diffusion of the fixing fluid.



## AUTOPSY AND STAINING METHODS

is preferred. This should be fixed in buffered formalin (pH 7) or alcohol formalin embedded in paraffin sectioned stained for iron with potassium ferrocyanide (Prussian blue reaction),<sup>4</sup> and counterstained with basic fuchsin. Grams of hemofuchsin or hemosiderin present may be readily demonstrated by this method. Gillman and Gillman<sup>11</sup> recommend a fresh mixture of equal parts of 2 per cent potassium ferrocyanide and 5 per cent hydrochloric acid in 70 per cent alcohol. The mixture is heated to boiling before use. Staining is carried out for one to two minutes followed by counterstaining with 1:6000 basic fuchsin.

## METHODS

Gomori's Chrome Alum Hematoxylin<sup>12</sup>

Fix thin slices in any aqueous fixative Bouin's fluid or formalin sublimate (formalin 1 part saturated mercuric chloride 3 parts water, 3 to 10 parts) are preferable. Embed in paraffin or celloidin. Run thin paraffin sections through xylene and alcohols to water. They should be protected by dipping the slide in a dilute (0.5 to 1 per cent) solution of collodion in alcohol-ether before the last alcohol. Stain celloidin sections floating (unattached). Remove if necessary mercury precipitates with Lugol's solution.

1. Treat sections with Bouin's solution containing 3 to 4 gm. of chrome alum per 100 cc. at 37° C. for twelve to twenty four hours. Wash under the tap until the color of picric acid is completely gone.

2. Oxidize sections with the following mixture for one to two minutes. 2.5 per cent solution of potassium permanganate and 5 per cent solution of sulfuric acid 1 part each distilled water 6 to 8 parts. Rinse.

3. Bleach with a 1 to 3 per cent solution of sodium bisulfite or oxalic acid. Wash under the tap.

4. Stain in following solution for about five to ten minutes or longer until  $\beta$  cells stand out dark blue against a much paler background. Mix equal volumes of 1 per cent aqueous hematoxylin and 2 per cent chrome alum solutions. Add to each 100 cc. of the mixture 2 cc. of 5 per cent potassium bichromate and 1 cc. of 5 per cent sulfuric acid. The mixture is ripe after forty-eight hours and will keep in the icebox for many months. It should have a deep opaque somewhat purplish blue shade. Filter before use. (Ripening can also be achieved by bringing the mixture to a short boil.)

5. Differentiate in 0.5 per cent hydrochloric acid alcohol for about one half minute. Wash under the tap for two to three minutes.

6. Counterstain with 0.5 per cent aqueous solution of phloxin for two or three minutes. Rinse.

7. Immerse in a 5 per cent solution of phosphotungstic acid for one to two minutes.

8. Wash under the tap for five minutes. The section should regain its red color.

9. Differentiate in 85 to 90 per cent alcohol until  $\alpha$  cells stand out deep red.

10. Transfer to 95 per cent and absolute alcohols clear in xylene and mount in balsam.

Result  $\beta$  cells deep blue  $\alpha$  cells bright red. (See Plate III.) The D cells are not distinguishable being red like  $\alpha$  cells. Also the  $\alpha$  cells do not always stain well in human pancreas. Dr. Sergio Benicome<sup>13</sup> has obtained very beautiful results in animal material with a modification which consists essentially in staining with ponceau fuchsin after step 5 rinsing in 1 per cent acetic acid differentiating in

1 per cent phosphomolybdic acid placing again in 1 per cent acetic acid for one minute then dehydrating in 3 changes of absolute alcohol. He uses a modified Zenker formal for fixation.

### Gomori's Aldehyde Fuchsin\*

This stain under proper conditions gives brilliant results imparting a deep purple shade to the granules of the  $\beta$  cells. (See Plate III.) Elastic tissue and mast cells are also stained. Almost any fixative may be used but formalin, formal-sublimate and Bouin's fluid are preferred.

3. Rinse in water followed by alcohol 70 per cent.

4. Stain fifteen to thirty minutes or longer even overnight in a Coplin jar filled with aldehyde fuchsin.

The dye is prepared as follows: add 1 cc. concentrated hydrochloric acid and 1 cc. of paraaldehyde U.S.P. to 100 cc. of a 0.5 per cent solution of basic fuchsin in 60 to 70 per cent alcohol. The color changes gradually (in about twenty-four hours at room temperature) to a deep purple almost indistinguishable from that of gentian violet and is then ready for use. Gomori states that solutions keep for about four weeks at room temperature and for over six months in the icebox. Fresh solutions stain faster than old ones. The stain may be rinsed off with alcohol and the slides inspected during staining.

5. Rinse in several changes of alcohol (concentration not stated) and counter stain as desired. A trichrome method such as Gomori's or Mission's<sup>1</sup> containing a green not a blue dye is satisfactory. Here again difficulty may be encountered in staining the  $\alpha$  cells. The D cells are said to take the green of the counter stain.

### Bensley's Modification<sup>2</sup> of Mallory's Aniline Blue Stain

1. Altmann's method: (a) Fix in Bouin's fluid or (b) soak thin pieces of formalin fixed tissue in

5. Dehydrate in alcohols, clear and mount.

### Terbrüggen's Modification<sup>3</sup> of Bensley's Acid Fuchsin—Methyl Green

1. Either (a) fix in Bensley's fluid or (b) soak thin pieces of formalin fixed tissue in 3 per cent potassium bichromate for a week.

2. Stain paraffin sections two hours in a 1:100 per cent dilution of a saturated (about 12 per cent) solution of acid fuchsin.

3. Differentiate for one-half to one minute in 2 per cent aqueous methyl green.

4. Dehydrate rapidly, clear and mount.

We have had occasional good results with this method. The  $\alpha$  cells are red,  $\beta$  cells bluish violet.

### Groschultz's Silver Impregnation

This important method originally intended for nerve fibers and popularized by Ferner,<sup>4</sup> has been subjected to extensive study by Hultquist and co-workers.<sup>5</sup> Their modification is given here.

Blocks of pancreas not more than 4 to 5 mm. thick are fixed 6 hours or more post mortem in 10 per cent formalin. Tissue from experimental animals (freshly killed) must be rolled in a piece of skin to protect from drying and kept at room temperature for several hours.

1 Make frozen sections receive in distilled water, wash twice in fresh distilled water. Sections must remain at least fifteen minutes in distilled water.

2 Very fatty pieces of pancreas are treated twice for fifteen seconds in pure pyridine. Then wash in fresh distilled water until water is entirely free from odor.

3 Sections are transferred with a glass rod to 20 per cent silver nitrate. The

material. It is about one to five hours for rats, fetuses and newborns about three to twelve hours for adults.

4 Sections are put in aqueous formalin diluted 1 to 4 in a series of dishes and changed until no more white clouds come off. They should not be in formalin over 10 minutes.

5 Sections are transferred to ammoniacal silver nitrate (see below) made as in the original method except that not a single drop of ammonia beyond that neces-

1 (say)  
drop  
it a lid  
stant  
note 1

fusely black.

6 Sections are transferred to dilute ammonia (2 parts ammonia and 8 parts water) and mounted in nitric acid, in chloride, in gold, carried out to be in

herent in all silver methods; however, with a little practice, as is reproducible results can be obtained.

#### Hultquist and Tegner's Modification<sup>14</sup> of Mallory's Phosphotungstic Acid Hematoxylin

- 1 Cut frozen sections of formalin fixed tissue and receive in distilled water.
- 2 Place sections in 0.5 per cent potassium permanganate for one to one and one-half minutes.

- 3 Bleach in 0.25 to 0.5 per cent sodium bisulfite (one-half to four minutes)
- 4 Wash quickly in 3 changes of distilled water
- 5 Treat for three to four hours in a 2 to 5 per cent solution of ferric ammonium sulfate

clouds disappear

the same as the method used by Gomori<sup>3</sup> for paraffin sections of Bouin fixed pancreas

### Friedenwald's<sup>17</sup> Method for Whole Mounts of Retina

The retina may be dissected out from the eye after fixation in formalin or other fixative and may be recovered from tissue already embedded in paraffin by melting the block in the oven and carrying the tissue back through xylol and alcohols to water. In either case the delicate translucent retina should be carefully separated from the deeply pigmented choroid. It is washed in tap water for twenty-four hours then in distilled water.

- 1 Place the retina (usually conveniently cut into about four pieces) in Hetchkiss' solution

3 Place in reducing rinse for twenty minutes. (Dissolve 1 gm. potassium iodide and 1 gm. sodium thiosulfate pentahydrate in 20 cc. distilled water. Add with stirring 30 cc. ethyl alcohol then 0.5 cc. 2 N hydrochloric acid. A precipitate of sulfur slowly forms and is allowed to settle out but the solution may be used immediately.)

- 4 Wash in distilled water thirty to sixty seconds

more 2 N hydrochloric  
mixture spontaneously  
Preserve in the dark

in two changes of absolute alcohol for ten minutes each, clear in xylol and mount in balsam, nerve-fiber layer up.

The capillary walls and microaneurysms are brought out with remarkable clarity (See Fig. 87 and Plate III.)



# UTOPSI AND STAINING METHODS

## Biggart's Pyrrhol Blue Eosin Method for Pituitary

- 1 Fix in Zenker formal eighteen to twenty four hours
  - 2 Stain thirty to thirty five minutes at 50° C in a mixture of equal parts of 0.5 per cent aqueous eosin Y and 0.3 per cent pyrrhol blue (isamine blue)
  - 3 Differentiate in the following solution 5 per cent sodium carbonate 10 parts absolute alcohol 40 parts (Differentiation is often quite rapid and must be watched carefully)
  - 4 Dehydrate quickly clear and mount in a neutral medium
- Result Acidophil cells red basophilic deep blue chromophobes light blue

## OTHER HISTOCHEMICAL METHODS

Most of the important new methods for enzymes and the like are cited in Pearse's review<sup>22</sup> and are described in detail by Gomori.<sup>21</sup> Other references on the mucopolysaccharides are given in Chapter 28

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	Deaths	% of all cases	Deaths	Deaths	% fall cases	Deaths	Deaths	% of all cases	Deaths	Deaths	% of all cases	Deaths	Deaths	% of all cases	Deaths
1	376	100 0	836	747	100 0	4001	100 0	100 0	3161	100 0	100 0	2 04	100 0		
2	108	63 8	347	208	24 6	2700	54 2	8 4	98	3 1	43	1 9			
3	57	17 5	208	203	24 3	2184	53 8		2047	65 0	1612	50 1			
4	57	17 5	203	83	9 9	1906	9 7		2035	64 6	1606	50 4			
5	11	3 4	32	32	3 8	100	4 7		140	4 4	1051	46 8			
6	9	2 8	41	41	4 9	381	9 4		368	11 7	161	7 0			
7	12	3 7	35	35	4 2	723	8 0		160	5 1	71	3 1			
8	5	1 5	12	12	1 4	84	2 1		80	2 5	41	1 8			
9	0	0 0	3	3	0 4	16	0 4		12	0 4	10	0 4			
10	24	7 4	106	106	12 7	557	13 7		345	10 9	153	6 7			
11	14	4 3	64	64	7 7	291	6 9		197	6 2	93	4 1			
12	0	0 0	6	6	0 7	21	0 5		3	0 1	0	0 0			
13	0	0 0	4	4	0 5	20	0 5		17	0 5	5	0 2			
14	2	0 6	3	3	0 4	25	0 6		16	0 5	2	0 1			
15	6	1 8	13	13	1 6	40	1 0		16	0 5	0	0 0			
16	0	0 0	1	1	0 1	20	0 9		20	0 6	11	0 8			
17	0	0 0	7	7	0 8	59	1 4		28	0 9	8	0 4			
18	0	0 0	8	8	0 9	25	1 9		47	1 5	22	1 1			
19	5	1 5	32	32	3 8	353	8 7		27	8 8	105	8 9			
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21	8	5	46	46	6 7	140	3 0		99	3 1	61	2 7			
22	0	0 0	7	7	0 8	54	2 1		5	1 8	41	1 0			
23	1	0 3	15	15	1 8	6	0 1		0	0 0	0	0 0			
24	1	0 3	-	-	0 2	27	0 7		1	0 3	11	0 6			
25	1	0 3	-	-	0 0	5	0 2		10	0 4	7	0 3			
26	6	1 8	21	21	2 5	11	4 9		119	3 8	113	4 9			

# APPENDIX C

## CAUSES OF DEATH IN 818 DIABETIC AUTOPSES\*

	to 1924	1924 to 1930	1930 through 1937	1937 through 1948	Totals	
					Number	Per cent
Total cases	105	157	224	332	818	100.0
A Coma present	66	32	29	9	136	16.6
B Coma absent	39	125	195	323	682	83.4
1 Cardioresenal vascular lesions						
a Arteriosclerotic					268	32.8
1 Aneurysm	0	0	1	1		
2 Cardiac	7	27	49	117		
3 Cerebral	2	5	12	10		
4 Gangrene	4	6	1	0		
5 Mesenteric	0	0	6	2		
6 Renal disease, vascular	0	3	2	13		
b Renal disease nonvascular					5	0.6
1 Glomerular	0	1	2	1		
2 Tubular	0	1	0	0		
2 Infections					213	26.0
a Gas gangrene	1	1	0	0		
b Meningitis	0	2	2	2		
c Multiple abscess	3	7	2	3		
d Pericarditis	0	2	4	0		
e Peritonitis	1	6	5	9		
f Pneumonia						
1 Bronchial	4	12	9	13		
2 Lobar	2	4	2	2		
g Pyelonephritis	0	1	1	11		
h Rheumatic fever	0	0	1	1		
i Septicemia	3	12	30	35		
j Syphilis	0	1	0	0		
k Tuberculosis	6	5	4	2		
l Empyema	0	1	0	1		
3 Cancer	0	13	26	42	81	9.9
4 Hemochromatosis	1	1	2	3	7	0.9
5 Pulmonary embolus	0	4	12	17	33	4.0
6 Insulin reaction (hypoglycemia)	0	0	3	1	4	0.5
7 Accident	0	1	1	0	2	0.2
8 Other causes	5	9	18	17	49	6.0
9 Undetermined				20	20	2.4

## INDEX

1

An-les nyo a l al 16  
 pa ca b  
 i d m 116 f  
 k line eff to 118  
 ja hoog al chang a lue to 11  
 A na rel h ology of 19  
 i po le in 12  
 A n n eular h po heu 12  
 A

Avec les honneurs de la Légion d'honneur

medial 134  
Mönche t pe 134  
o gans affe ed n d be 4  
pan ea e 6.  
abb les on 142  
renal 169  
roen gen a d sign of 31  
va s u m o 141

Allen gland      otal      unore of and

kl ou 201  
 l be em l u an l 201 f 2 8  
 m n hed fu on 207  
 e pe n n al d abe 244  
 l pe l as a f 201  
 n n fa te 25  
 n edu a 204  
 phen chromy om 201  
 n l i te 12 2 5  
 n m n 2  
 renall one n l i

Alpha cell hono e 2'5  
An i i rela n to h al n 31

An l i t r e a n t o n a l n 33  
 Am lo do 2  
 Ang na p e o 161  
 An mal d be s n 11 14 1  
 Aorta a e o l o f 132  
 A u s n d n e of 181  
 A r o l a s l w 15 161  
 A o n o n 12 ff  
 a a n i a l a w a i 14  
 I f i a n n 131 144  
 a 134 139  
 I 1 140 143  
 I a l a g r a f 14 112  
 n n a 8 ff  
 I d u 158 159 1 n  
 I e c n of 131  
 I l n o 141  
 w l n f 153  
 w 14  
 o k of 14  
 f r i p u of n a f l g l u a o n  
 21

21  
 1 le 148  
 e gre e and 151  
 il pe e and 10  
 n bl on the 1st 183  
 n d nee in the e n 118 140  
 n e and 146  
 i d in 140  
 i popro e 14

B L O N F Y U R E

gang ene 125  
Roue p g n e n t a u f 100  
B a n h a n g e t n n u l n r s 211  
oma pa h o l o g 120  
k u o u e u l a n o n b 211  
gl o g e o n e a f  
les ons of 11  
Bronzo d a b e e s 20  
Bu e n d e r e 15

( 4 7 nov of h and vowel 31 1  
of d of Lang 11 on 39  
of sk 10

( ara chem al ul - of 181  
ju n i a b e s 181  
i r e a l n e s s 181  
( a s e s of d a t 211 211 211 211  
( n r o - a n e 19  
r e l a t i o n s h i p 138 11  
h e n o t h e r g e 191  
( l i d r e n c a u s e s of d a b i n d a b e 211  
h e r e d i t a r y d i s e a s e u s i n g 10

Children hyaline degeneration in  
 of 213  
 hydropic degeneration in 213  
 lymphocytic infiltration of pancreas  
 213  
 pathology of diabetes in 210 ff  
 vascular disease in 215  
 Cholelithiasis See Gall stones  
 Cholesterol arteriosclerosis and 143  
 xanthomas and 103  
 Choline 113  
 Chylomicron count 120 145  
 Clear cells 26  
 Collagen 146 318  
 Collagen swelling of 146 318  
 Coma 116 ff  
 abdominal distention in 119  
 brain in 120  
 cause of death as 329 330  
 gastrointestinal hemorrhage in 119  
 hypoglycemic 311  
 leukoencephalitis in 120  
 hypemia in 120  
 pancreatic lesions in 118  
 pancreatitis and 120  
 pathological findings in 116

occlusion 150  
 sclerosis 158 ff  
 thrombosis 158

Cortisone diabetes 45 47 284 299  
 Cystine 292 293  
 Cysts pancreatic 71

## D

DEATH causes of in diabetes mellitus 211  
 220 329 330  
 Dehydroascorbic acid 280  
 Dental caries progressive 126  
 Dermatitis gangrenosa 120  
 Diabetic nephropathy 178  
 neuropathy 192  
 retinopathy 183  
 Diet in hemochromatosis 229  
 effect on islets 22 303  
 Dog spontaneous diabetes in 11  
 Duct cells histology of 18  
 Ducts pancreatic anatomy of 16  
 metaplasia of 73

## E

ENDOCARDITIS rarity in diabetes mellitus  
 166  
 Endometrium cancer of 269  
 Enterochromaffin cells 26  
 Epidermoidalization of pancreatic ducts 81  
 Estrogens diabetogenic effect, 285

veins, 186  
 Fy elids 181

FAT glycogen and in liver 89  
 metabolism of 102 ff  
 in kidney 117 168  
 in skin 104  
 in spleen 102  
 Fatty infiltration of islands of Langerhans  
 53  
 of pancreas 64  
 Fibrin nets in retina 189  
 Fibrinoid change 318  
 Fibrosis interacinar 63  
 interlobular 63  
 of islands of Langerhans 40  
 in nondiabetics 76

## G

GALL bladder 107  
 disease in diabetes mellitus 107  
 stones analyses of 110  
 frequency of 107  
 Gangrene 151  
 collateral circulation in 151  
 diabetic 151  
 embolic 151  
 gas 125 151  
 moist 156  
 prevention of 156  
 Gas gangrene 151  
 simulation by Bacillus coli infection  
 125  
 Gingivitis 126  
 Glucagon 27 239 291  
 Glucose experiment al diabetes 283  
 Glutathione 292  
 Glycogen 83 ff  
 brain distribution in 97  
 coma depletion in 90  
 content of post mortem liver 314  
 deposition of effect of insulin on 89  
 distribution diagnostic value of 99  
 eye distribution in 97 181  
 fat in liver and 89  
 fixation of 84 314 322  
 heart, distribution in 85 252  
 islands of Langerhans 46 234  
 kidney distribution in 91, 93 168  
 infiltration of and glycours 93 95

## INDEX

- Glycogen leukocytes, deposition in 97  
 liver, distribution in 88  
 liver cell nuclear content, 88  
 liver cells, relation of 93  
 methods of demonstrating 121  
 muscle, voluntary, distribution in, 96  
 pancreas, distribution in 97  
 pituitary, distribution in 97  
 placenta, distribution in 97  
 post mortem disappearance of 314  
 preservation by formalin 314  
 skin content of 96  
 stains for, 121  
 storage disease, 99  
 Glycosuria, acromegaly and 193  
 adrenal tumor and 201, 203  
 emotional 191  
 kidneys, glycogenic infiltration in 168  
 pituitary and 195  
 Golgi apparatus, 11  
 Gomori's staining methods 121  
 (islands) 208  
 Gram-Schultz stain, 16 2; 26 5; 214 125  
 ground substances 146 117  
 Guinea pig C cells in 21  
 resistance to alloxan 276  
 Gummis of pancreas 61
- H
- Heart, absence of, 163  
 endocarditis, rarity in diabetes mellitus 166  
 fibrosis of 161  
 glycogen content of 8; 116  
 hypertrophy of 162  
 in infants 231  
 infarcts of 157  
 in youth 15; 161  
 weights of, 162  
 Hemochromatosis 226 ff  
 etiology of 224  
 insulin resistance and 211  
 pancreatic regeneration in 206  
 Hemifuscin 228  
 Hemorrhage, gastrointestinal in coma 119  
 into islands of Langerhans 44 51  
 Hemostatin 226  
 staining of 321  
 Henle's type glycogenic infiltration of 92  
 11 168  
 Hepatomegaly See Liver  
 Hereditary diabetes mellitus and 301  
 Hexokinase 82 301  
 Histidine, hyalination in islands 31  
 origin of 31  
 staining reactions of, 33  
 Histidine in membrane in lungs 250 251  
 Hyalination of islands of Langerhans age-  
 incidence of 39  
 amylase in 31 314  
 calcification of 79  
 frequency of, 39  
 in adenomas, 35  
 incidence of in cases of long dura-  
 tion 37  
 in nondiabetics 79
- Hyalination of islands, nature of 33  
 variation in degree of 35  
 Hydropic change 42 ff 291  
 children occurrence in 41  
 experimental diabetes occurrence in 274  
 fulminating diabetes occurrence in 41  
 glycogen in 46  
 post mortem change relation to 46  
 Hyperglycemia experimental 282 283  
 296 303  
 Hyperglycemic glycosolysis (H.G.)  
 factor 27 23; 294  
 Hypernatremia 210 ff  
 cerebral changes in 211  
 experimental 244  
 medico-legal significance of 311  
 tumors of islands and 211  
 Hyper-eosinophilia to insulin 218  
 Hyperthyroidism diabetes mellitus and  
 207  
 Hypoglycemia pathology 211  
 Hypophyseal diabetes 280  
 Hypophysis See Pituitary  
 Hypothalamus lesions of 191  
 Hypothyroidism hypertrophy of islands of  
 Langerhans in 209
- I
- Immunization theory of arteriosclerosis 129  
 137  
 Infant mortality 21  
 Infants of diabetic mothers 214 ff  
 Infection 122 ff  
 as cause of death 121 130  
 of diabetes 303  
 focal 126  
 fungus 124  
 gangrene and 156  
 lowered resistance to 126  
 urinary importance of 126  
 Insular hypothyroidism 7 9 11  
 Insulin allergy 216  
 antibodies to 235  
 atrophy 218  
 cerebral effects of 217  
 content of pancreas 302  
 deficiency anatomical causes of 291  
 electrocardiogram effect on 216  
 fish 11  
 glycogen deposition effect on, 161  
 hydropic degeneration effect on 44  
 hyperosmolarity to 216  
 hypodystrophy 216  
 liver glycogen, effect on 161  
 production of 11  
 reaction 311  
 cerebral changes in 217  
 need for autopsy in fatal 311  
 resistance 235  
 carcinoma of pancreas and 237  
 hemochromatosis and 236  
 liver injury and 234  
 pituitary disease and 218  
 thrombosis of hepatic artery and  
 1 Insulinase 234 301

Inter-capillary glomerulosclerosis 170 *ff*  
317  
clinical recognition 174  
experimental 173  
histogenesis 171  
nodular form 171

Iris 181

Islands of Langerhans 21 *ff*  
absence of 9

in nondiabetics 7

adenoma of 51 240

alpha cells 23 213

amyloid in 33

aplasia of 57

argentaffin reaction 2

beta cells 23

blood supply of 28

C cells 24

calcification of 31

capsule of 24

carcinoma of 241

D cells 24

development of 16

diet effect of 22 303

differential cell counts 28 *ff*

distribution of 21

fatty infiltration of 33

fibrosis of 40

in nondiabetics 7

glycogen in 46

granules specific of 23

stim for 324

Hydropic change of 42 *ff*

experimental production of 273 *ff*

in non-diabetics 9

hyperplasia of in infants 254

hypertrophy of 49

in adren. insufficiency 202

in infants 254

in nondiabetics 80

lymphocytic infiltration of 41 213

in nondiabetics 79

mitosis in 81 233

necrosis of 48

nerve supply of 28

normal in diabetes mellitus 22

number of 21 56

origin of 16

post mortem degeneration of 44

pyknotic nuclei in 48

in nondiabetics 79

qualitative changes in 31

quantitative changes in 21 22 33 *ff*

regeneration of 214 213

toxic injury to 122

transition from 27

volume of 22

## K

Kidney 116

Arterioles, arteriolar sclerosis in 169

Kidney, fat in 117 168

glycogen deposition in 91 93 168

in infants 258

injury of acidosis and 117

intercapillary glomerulosclerosis 170 *ff*  
317

mixed diabetic nephropathy 168, 178

necrotizing renal papillitis 176

pyelonephritis 175

## L

Lanus 182

Lapemia 102 120

Lipocytic 112

Lipochrome 106

Lipoid deposits in 102

Lipomatous 64 11

Lipoproteins 14

Liver enlargement of 111

fatty infiltration of 111

fluid content of 112

function tests 114

of non-diabetics 114

## M

Long duration cases 218 *ff*

Lymphocytic infiltration of islands of

Langerhans 41 213

## N

Nausea 44

Muscle voluntary glycogen content of 98

Myocardium abscess of 16

Myxodema diabetes and 20

## O

Nervous system central pathology of 131  
249

Nesidioblastoma 241

Neuritis peripheral 192

Neurogenic diabetes 299

Neuropathic diabetic 192

arteriosclerosis in 193

## O

Obesity 301

experimental 296

"Onkocytes," 21, 23  
Ovaries, atrophy of, 201  
in infants, 260

## P

PANINIAN corpuscles in pancreas, 28  
Pancreas, atrophy of, 61  
acinar cells, 19  
anatomy of, 72  
anatomical relations of, 15  
arteriole of, 65  
blood supply of, 17, 306  
ducts in, 64  
endocrine of, 72  
  insulin resistance, and, 237  
  relation to diabetes mellitus, 270  
  size of, 78  
development of, 17  
ducts, 18  
etiology of diabetes, and, 290  
fatty infiltration of, 64, 77  
fibrosis of, 51  
function of, 322  
glycogen in ducts of, 91  
guinea, 61  
hemochromatosis, effect on, 228  
histology of, 17  
insulin content, 302  
islets of, in nondiabetics, 76 ff.  
lipomatosis of, 64, 77  
metastatic tumors of, 78  
nervous supply of, 28  
normal, 16 ff.  
regeneration of, 214, 215  
size of, 73  
stains for, 321  
syphilis of, 61  
tuberculosis of, 78  
weight of, 13, 71  
Pancreaticoduodenal, 274  
  experimental, 274  
Pancreatic ducts, 18  
  glycogen in, 91  
Pancreatitis, 67, 293  
  acute and, 120  
Parathyroid gland, 206  
Periodic acid, Schiff reagent method, 171,  
  181  
Periductalitis, 126  
Pituitary gland, 186  
  basophilic cells, 197  
  cancer and, 270  
  chromophobe cells, 197  
  diabetes mellitus and, 195 ff., 297  
  disease, insulin resistance and, 216  
  experimental diabetes, 289  
  glycogen deposition in, 97  
  hypertrophy and, 195  
  growth hormone, 281  
  actions in, and diabetes, 196, 197

Placenta, glycogen distribution in, 97  
  lipoids in, 262  
  weight of, 262  
Pneumonia, 124  
  as cause of death, 329, 330  
Polysaccharides, 83 ff., 93, 111  
Post-mortem blood sugar, 312, 314  
  diagnosis of diabetes, 112  
Potassium, 121, 166  
Pregnancy, 243, 264  
Prematurity, 251  
Pruritus, infection resulting from, 97  
Psychogenic origin, 301, 310  
Pyelonephritis, 175  
  renal papillitis, 176  
Pyogenic infections, 122  
Pyorrhea, 126

## R

Refractive changes in diabetes, 181  
Regeneration of pancreas, 214, 215  
Reischoffen-Endothelial cells, lipid infiltration  
  of, 102  
Retina, microaneurysms of, 181  
Retinitis proliferans, 184  
Retinopathy, diabetic, 181  
Roentgenological methods, 141, 162, 163,  
  165

"Rusakov cells," 16, 25, 26, 34, 214, 221  
Skin, Bullous cut infection of, 97  
  calcification of, 107  
  gas gangrene, stimulation in, 125  
Spinal cord, changes in, 191  
Spleen, lipid deposits in, 109  
Splanchnic artery, arteriosclerosis of, 65  
Stains, 17, 121  
Staphylococcus albus, significance of, 121  
Sterility in male, 208  
Stomach, distention of, 114  
Streptococcus infection, 125  
Stress, and diabetes, 301, 301  
Sulphydryl compounds, 242, 299  
Syphilis, death due to, 127  
  pancreatic changes in, 69, 127

## T

Thrombo-embolism, diabetes, 115  
Thrombosis, cerebral, 191  
  coronary, 137  
Thymus, 208  
Thyroid, 208 ff., 299  
Thyroid diabetes, 242, 299  
Transition theory, 12, 21, 26, 28  
Traumatic diabetes, 301, 310  
Treatment, in relation to complications,  
  218, 225  
Tuberculosis, 127  
  of pancreas, 78



- Intercapillary glomerulosclerosis 170 *ff*, 317  
 clinical recognition 174  
 experimental, 173  
 histogenesis, 171  
 nodular form, 171
- Iris** 181
- Islands of Langerhans** 21 *ff*  
 absence of 9  
   in nondiabetics 79  
 adenoma of 31, 210  
 alpha cells 23, 203  
 amyloid in, 33  
 aplasia of 57  
 argentaffin reaction 25  
 beta cells 21  
 blood supply of 25  
 C cells 24  
 calcification of 31  
 capsule of 27  
 carcinoma of 241  
 D cells 24  
 development of 16  
 diet effect of 22 303  
 differential cell counts 58 *ff*  
 distribution of 21  
 fatty infiltration of 31  
 fibrosis of 40  
   in nondiabetics 79  
 glycogen in 46  
 granules specific of 21  
   stain for 324  
 hemochromatosis regeneration in 233  
 hemorrhage into 31  
 hyaline change of 31 *ff*  
   in nondiabetics 79  
 hydropic change of 42 *ff*  
   experimental production of 274 *ff*  
   in nondiabetics 79  
 hyperplasia of in infants 234  
 hypertrophy of 49  
   in adrenal insufficiency 202  
   in infants 234  
   in nondiabetics 80  
 lymphocyte infiltration of 41 213  
   in nondiabetics 79  
 mitosis in 81 233  
 necrosis of 48  
 nerve supply of 28  
 normal in diabetes mellitus 52  
 number of 21 56  
 origin of 16  
 post mortem degeneration of 44  
 pyknotic nuclei in 48  
   in nondiabetics 79  
 qualitative changes in 31  
 quantitative changes in 21 22 35 *ff*  
 regeneration of 214 213  
 toxic injury to 122  
 transition from veins 27  
 volume of 22
- K**
- KETONURIA**, 116
- Kidney**, arteriolar sclerosis in 169
- L**
- Latent, 162
- Lipemia, 102 120
- Lipocac, 112
- Lipochrome 106
- Lipoid deposits spleen 102
- Lipomatosis 64 77
- Lipoproteins 14
- Liver**, enlargement of 111  
 fatty infiltration of 111  
 fluid content of, 112  
 function tests 114  
 glycogen amount of 88  
 role in diabetes 300  
 weight of in diabetes 111
- Liver cell nuclei** glycogenic vacuolization of, 88
- Long duration cases, 218 *ff*
- Lymphocytic infiltration of islands of Langerhans 41 213
- M**
- Mumps 42
- Muscle voluntary glycogen content of
- Myocardium abscess of 16
- Mixedema diabetes and 207
- N**
- Nervous system central pathology of 19 299
- Nesidioblastoma 241
- Neuritis peripheral 192
- Neurogenic diabetes 299
- Neuropathy diabetic 192  
 arterioleclerosis in 193
- O**
- OBESITY** 301  
 experimental, 296



Tumors, metastatic of pancreas 78  
Types of diabetes 302

## U

UNIDRYN, concept 316  
Uric acid diabetes 285 291

## V

VASCULAR disease 120 ff  
Veins of retina 186  
Vernix membrane 250 253  
Vitamin C in alpl<sup>a</sup> cells 26

von Gierke's disease 99

## X

X RAY in vascular disease 131  
Xanthelasma 181  
Xanthemia 106  
Xanthoma cholesterol content of 103  
diabeticorum 104  
Xanthomatosis generalized 103

## Z

ZINCOCY granules 20

